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# MOLECULAR IDENTIFICATION OF MICROBIAL CONTAMINATIONS IN THE FITNESS CENTER IN MAKKAH REGION

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## Abstract

As the condition increases and seeks to remain healthy, the number of people who plan to join a fitness center or "gym" has increased markedly. From where this individual does understand, the study of the variety of bacteria showing the stolen has led him to care for people, with a popular fitness center located in the province of Makka, Saudi Arabia. Different bacteria must be eliminated from other gyms in Makkah, Saudi Arabia, in a total of three areas 46 two sports equipment. Both types and characteristics of bacteria, while some have been tested in hemolytic surgery for antibiotic resistance. Corynebacterium antibiotics in different forms did not react the same; however, isolates tested for M17 and N12 showed the greatest resistance to antibiotics. Furthermore, sixteen bacterial strains of human blood β-agar displayed high hemolytic activity. In the gym isolates 2 (9 strains) followed in gym 1 (7 modes), B row hemolytic activity was highest. It is important to note that gram-positive bacteria were positive in all kinds of ways, and catalase was positive. Six strains belonging to the genus Bacillus, Brachybacterium, Geobacillus, Microbacterium, Micrococcus, and Staphylococcus and other pathogenic bacteria were known as possible individuals to use the morphological, biochemical, and rRNA gene of the 16S series. In general, this research illustrates the health and fitness centers in the individual being studied and the risks that are considered necessary to periodically study possible microbial contamination in the mixture in the gym to ensure people's protection.

**Keywords:** 16S rRNA Gene. Fitness Center. Hemolytic Activity. Microbial Infection.

## 1. Introduction

There has been a remarkable rise in the number of people choosing to join a fitness center or 'gym' with growing public aspiration to keep fit and safe. While many studies focusing on environmental hygiene and sanitation have reported a direct connection between microbial load in the local environment and the risk of transmission of a pathogen (Oliver et al. 2005; Cooper 2013). Various facilities that are regularly in contact with tourists can be hotspots for various pathogenic microorganisms, such as antibiotic-resistant bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA) (Montgomery et al. 2010), vancomycin-resistant enterococci (VRE) (Ramadhan and Heqedus 2005; Ahmed et al. 2020), etc. To obtain a better understanding of the risk associated with pathogen transmission from such facilities, an understanding of the overall bacterial population and diversity in fitness centers is needed. Most research performed at gymnasiums, playgrounds, sports fields, or locations where people meet others to date are mainly focused

on staphylococci transmission, (David et al. 2008; Gorwitz 2008; Ryan et al. 2011) and are relying on culture-based techniques (Goldhammer et al. 2006). However, the global diversity of microbial communities associated with the ecosystem of fitness centers remains largely unknown because of the difficulty of increasing many microorganisms (Pace 1997; Mukherjee et al. 2014).

Interestingly, it is now possible to research the microbial population from different sources in more detail with the help of modern high-throughput sequencing techniques and various bioinformatics tools (Agrawal et al. 2015). Microbial populations have been examined in some indoor settings, such as office buildings (Hewitt et al. 2012), public bathrooms (Flores et al. 2011), and hospitals (Kembel et al. 2012), where the primary source of bacteria in human skin. Several studies (Abad et al. 1994; Bures et al. 2000; Brooke et al. 2009; Zhang et al. 2012) have identified surfaces as possible sources for the transmission of infectious microorganisms in public places such as computers, telephones, headsets, tables, automated teller machines (ATM), cash machines, elevator buttons, etc. Overall, these experiments showed that microbial pollution hosts comparatively diverse microbial communities dominated by human-associated bacteria on different surfaces and could be possible sources of bacterial pathogens that could easily be transmitted from person to person by merely touching the surfaces. Therefore, to determine the prevalence of pathogenic bacterial strains in the areas studied, it is important to research the bacterial diversity in different surfaces where people are normally visited, such as fitness centers, markets, hospitals, public libraries, etc., and characterize and classify the bacteria.

Gyms offer a specific setting to explore the diversity of microbial communities relative to other indoor environments because of the physical activities with a high level of surface contact by individuals with various personal hygienic habits (Mukherjee et al. 2014). Little information on the microbial ecology of fitness centers located in Saudi Arabia is available so far, however. Therefore, the present study aims to distinguish various bacterial strains from different surfaces of selected gyms located in Mecca, Saudi Arabia (e.g., dumbbells, bars, and walking machines); to assess the ability of bacterial resistance against several antibiotics; to determine the hemolytic activity of bacteria and to classify and identify isolates based on their hemolytic activity.

## 2. Material and Methods

### Sample selection and bacteria isolation

In January 2017, surface swab samples were obtained from two (2) membership-based gyms located in Makkah, Saudi Arabia. Samples from treadmills (handles), dumbbells (whole surface), and bench press bars (stems) that were not sanitized by cotton-tipped swabs (Sanicult TM, Thermo Remel/ Starplex Science Inc., Etobicoke, ON, Canada) according to the method defined by Mukherjee et al. (2014) before sample collection. Samples were then held in an icebox and transported within three hours (h) of the collection to the laboratory.

For the isolation of bacteria, precisely 100 µl of an aliquot from each sample was spread on plates of nutrient agar (NA) and incubated for 48 h at 37ºC. Repeated streak culture on the same medium distilled morphologically different colonies. The purified bacterial isolates were then deposited at -80º C for further analysis in a 25 percent glycerol solution.

### The isolated bacteria antibiotic susceptibility test

The Kirby-Bauer disk diffusion method on NA was conducted in conjunction with the bacterial antibiotic susceptibility test (Bauer et al. 1966). Twelve (12) antibiotic discs were purchased from Mast Group Ltd, Merseyside, UK, consisting of penicillin G (10 units), erythromycin (15 µg), ampicillin (10 µg), cephalothin (30 µg), clindamycin (2 µg), cotrimoxazole (25 µg), gentamicin (10 µg), vancomycin (30 µg), fusidic acid (10 µg), chloramphenicol (30 µg), oxacillin (1 µg) and cefepime (30 µg). Each isolate was spread over the plates-containing antibiotic discs and incubated at 37 oC. The clearance zones (inhibition zone) around the antibiotic discs were measured using a meter ruler after 48 h of incubation and the isolates were graded as resistant, intermediate, and sensitive.

**Blood agar hemolytic operation of bacteria and MacConkey agar bacterial growth**

Increases in bacterial isolates have also been observed at 37º C for 48 h on human blood agar media to detect bacterial hemolytic activity. Instead of sheep blood, human blood agar was used because of its ready availability. All the isolates were grown on MacConkey agar (Oxoid Ltd., UK) for 48 h at 37º C to selectively isolate and distinguish gram-negative bacteria based on lactose fermentation. The presence of bile salts and crystal violet dye in the media prevents the growth of most gram-positive bacteria, and the transition from pink to neutral red dye suggests the ability of bacterial strains to ferment lactose.

**Bacterial isolate morphological and biochemical characterization**

**Characterization of anatomy**

Bacterial isolates were grown for 48 h at 37º C on separate NA plates and their morphological characteristics were recorded, such as colony shape and color.

**Characterization of biochemicals**

**Staining of grams**

All isolates are further described by their gram staining properties. A drop of bacterial suspension is taken and air dried to make a stain on the glass slide. With the aid of flame fire, the smear was then fixed and stained with crystal violet solution for 1 min. Cleaning with distilled water followed. After that, add a gram of iodine for 1 min.

In addition, the purified water was washed and air-dried. The smear was then washed for 1 min with 95 % ethanol. Cleaning with distilled water followed. Finally, for 1 min, the lie was counter-stained with safranin. In addition, the purified water was painted and air-dried. Then the slide under the microscope was examined. What occurs in the pink color of the colony is known as gram-negative bacteria (Cerny 1976).

**Test with catalase**

Bacterial cultures were grown on NA plates at 37º C for 24 h to assess the catalase function. To observe the formation of gas bubbles that signify a positive reaction (Hayward 1960), a loop of each bacterial culture was mixed with a drop of 3 percent of hydrogen peroxide ($H_2O_2$) on a clean glass slide.

**Molecular identification of bacteria through 16S rRNA gene sequencing and phylogenetic analysis**

**Genomic DNA extraction**

Following the manufacturer's guidelines, the extraction of bacterial genomic DNA was carried out using the QIAGEN Kit. Briefly, bacterial isolates were propagated in 5 ml of nutrient broth (NB) overnight and 1.75 ml of each strain was transferred to the microcentrifuge tube followed by centrifugation for 5 min at 13000 rpm. 180 µl of enzymatic lysis buffer was added and the tube vortexed for 10-20 s after decanting the supernatant. After incubation, for 30 min. 25 µl proteinase K and 200 µl alkaline lysis (AL) buffer were added at 37º C and vortexed briefly. The mixture was then incubated for 30 minutes at 56º C. The solution was transferred to a mini spin column supplied by the manufacturer after the addition of 200 µl of 100 percent ethanol and centrifuged for 1 min at 13000 rpm. The filter was subsequently discarded. 500 µl of AW1 wash buffer was added to the column and centrifuged for 1 min at 13000 rpm. After that, wash buffer AW2 of 500 µl was added to the column and centrifuged for 3 min at 13000 rpm. Then the filtrate was discarded. Nuclease-free water (100 µl) was finally added to the column and centrifuged for 1 min at 13000 rpm. Samples of DNA are then stored at -20º C.

**Primers for 16s of rRNA gene amplification, sequencing, and phylogenetic analysis**

Universal primers were used to amplify the 16S rRNA gene, 27F (5 -AGAGTTTGATCCTGCTCAG-3) (for all isolates), 1445R (5 -AAGGAGGTGATCCAGCCGCA-3) (for the following strains: M1, M3-M4, M8, M10, M12-M13, N1, N3-N4, N8, N10, N12-14, N23-N27) and 511R (5 -GCGGCTGCCACRKAGT-3) (for the following strains: M2, M5-M7, M9, M11, M14).

**Protocols for PCRs**

Following the manufacturer's guidelines, the Thermo Scientific PCR master mix was used to amplify the DNA. Amplification was carried out at 94º C for 5 min in a thermocycler (Mastercycler ® Gradient, Eppendorf, Hamburg, Germany), followed by 32 cycles of 45 s at 94 oC, 45 s at 60º C and 90 s at 72º C, with a final extension of 10 min.

**Amplified PCR product analysis**

A 3 µl aliquot of each PCR amplicon was electrophoresed and visualized under a UV transilluminator (BioDoc-IT system, Japan) on a 1 percent agarose gel containing ethidium bromide in 1X Tris-Acetate-EDTA (TAE) buffer at 120 V for 40 min.

**Sequencing amplified PCR amplicons**

Amplified products were purified using the QIAquick PCR purification kit (Promega, Madison, WI, USA) and sequenced with an ABI Prism ® 310 Genetic Analyzer (Applied Biosystems) using the Big Dye terminator cycle sequencing Ready Reaction kit (Applied Biosystems, Forster City, CA, USA).

**Analysis with phylogenetics**

SnapGene Viewer software version 3.3.3 manually edited the sequences and then compared them with the GenBank NCBI database (http:/www.ncbi.nlm.nih.gov) using BLASTN search, and the reference sequences were retrieved for phylogenetic analysis. Using the MEGA available on the NCBI website, phylogenetic trees were constructed.

**3. Results**

The present study was conducted to explore the diversity of culturable bacteria in two (2) selected fitness centers located in Mecca, Saudi Arabia; to screen them for potential pathogenic strains, and to identify them based on gene sequences of their 16S rRNA. The following sub-sections present the experimental results of the research presented in this chapter.

**Bacteria isolation**

The isolated bacteria's antibiotic susceptibility Blood agar hemolytic activity of isolated bacteria and MacConkey agar bacterial growth.

**Identification of separate strains of bacteria**

**Bacteria isolation**

A total of forty-six (46) bacterial strains were isolated from two selected fitness centers and purified on nutrient agar (NA) medium by repeated streak culture. A total of nineteen (19) strains from gym 1 were isolated and twenty-seven (27) strains from gym 2 were isolated (Table 1). The highest number of bacteria, however, was found on Gym 2 treadmills (13 strains) followed by Gym 1 treadmills (8 strains). In addition, the lowest number of bacteria was recorded from gym 1 (5 strains) bench press bars, followed by gym 1 (6 strains) dumbbells. Bacteria isolated from different gym facilities were named M1-M19 and N1-N27 from gym two (Table 1).

**Table 1.** List of bacteria with their source of isolation and gram staining.

| Bacterial Isolates | Source of bacterial isolation | Place of sample collection | Gram staining |
|---|---|---|---|
| M1 | Treadmills (handles) | Gym 1 | + |
| M2 | Treadmills (handles) | Gym 1 | + |
| M3 | Treadmills (handles) | Gym 1 | + |
| M4 | Treadmills (handles) | Gym 1 | + |
| M5 | Bench press bar | Gym 1 | + |
| M6 | Bench press bar | Gym 1 | + |
| M7 | Bench press bar | Gym 1 | + |
| M8 | Bench press bar | Gym 1 | + |
| M9 | Dumbbell (entire surface) | Gym 1 | + |
| M10 | Dumbbell (entire surface) | Gym 1 | + |
| M11 | Dumbbell (entire surface) | Gym 1 | + |
| M12 | Dumbbell (entire surface) | Gym 1 | + |
| M13 | Dumbbell (entire surface) | Gym 1 | + |
| M14 | Dumbbell (entire surface) | Gym 1 | + |
| M15 | Treadmills (handles) | Gym 1 | + |
| M16 | Treadmills (handles) | Gym 1 | + |
| M17 | Treadmills (handles) | Gym 1 | + |
| M18 | Treadmills (handles) | Gym 1 | + |
| M19 | Bench press bar | Gym 1 | + |
| N1 | Treadmills (handles) | Gym 2 | + |
| N2 | Treadmills (handles) | Gym 2 | + |
| N3 | Treadmills (handles) | Gym 2 | + |
| N4 | Treadmills (handles) | Gym 2 | + |
| N5 | Treadmills (handles) | Gym 2 | + |
| N6 | Treadmills (handles) | Gym 2 | + |
| N7 | Treadmills (handles) | Gym 2 | + |
| N8 | Treadmills (handles) | Gym 2 | + |
| N9 | Treadmills (handles) | Gym 2 | + |
| N10 | Treadmills (handles) | Gym 2 | + |
| N11 | Treadmills (handles) | Gym 2 | + |
| N12 | Treadmills (handles) | Gym 2 | + |
| N13 | Treadmills (handles) | Gym 2 | + |
| N14 | Dumbbell (entire surface) | Gym 2 | + |
| N15 | Dumbbell (entire surface) | Gym 2 | + |
| N16 | Dumbbell (entire surface) | Gym 2 | + |
| N17 | Dumbbell (entire surface) | Gym 2 | + |
| N18 | Dumbbell (entire surface) | Gym 2 | + |
| N19 | Dumbbell (entire surface) | Gym 2 | + |
| N20 | Dumbbell (entire surface) | Gym 2 | + |
| N21 | Bench press bar | Gym 2 | + |
| N22 | Bench press bar | Gym 2 | + |
| N23 | Bench press bar | Gym 2 | + |
| N24 | Bench press bar | Gym 2 | + |
| N25 | Bench press bar | Gym 2 | + |
| N26 | Bench press bar | Gym 2 | + |
| N27 | Bench press bar | Gym 2 | + |

**Antibiotic susceptibility of the isolated bacteria**

All the forty-six (46) bacterial isolates were tested against twelve (12) different antibiotics to observe their antibiotic sensitivity (Table 2) (Figure 1). Out of forty-six (46) bacteria, forty (40) bacteria were found resistant to at least one of the antibiotics tested (Table 2). Only six (6) strains, namely M8, M10, M12, N5, N9, and N25 were found susceptible to all the twelve (12) antibiotics tested. However, two (2) isolates (M17 and N12) showed resistance to a maximum of five (5) antibiotics out of twelve (12) antibiotics tested. Bacterial isolate M18 was found resistant to four (4) of the tested antibiotics. Among the tested antibiotics, cephalothin (30 µg) and vancomycin (30 µg) were found most effective against the bacterial isolates as all strains were found susceptible to these two (2) antibiotics. Clindamycin (2 µg) and gentamicin (10 µg) were effective against forty-seven of the isolated strains. On the other hand, oxacillin (1 µg) followed by Cotrimoxazole (25 µg) showed the lowest effectiveness against the tested bacteria.

**Table 2.** Antibiotic susceptibility of the isolated bacteria.

| Bacterial Isolates | Antibiotic susceptibility of the isolated bacteria* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PG | E | AP | KF | CD | TS | GM | VA | FC | C | OX | CPM |
| M1 | S | S | S | S | S | R | S | S | R | R | S | S |
| M2 | S | S | S | S | S | R | S | S | S | S | S | S |
| M3 | S | S | S | S | S | R | S | S | S | S | R | S |
| M4 | S | S | S | S | S | R | S | S | S | S | R | S |
| M5 | R | S | S | S | S | S | S | S | S | S | S | S |
| M6 | S | S | S | S | S | R | S | S | S | S | R | S |
| M7 | R | S | S | S | S | S | S | S | S | R | S | S |
| M8 | S | S | S | S | S | S | S | S | S | S | S | S |
| M9 | S | S | S | S | S | S | S | S | S | S | R | R |
| M10 | S | S | S | S | S | S | S | S | S | S | S | S |
| M11 | S | S | S | S | S | R | S | S | R | S | S | S |
| M12 | S | S | S | S | S | S | S | S | S | S | S | S |
| M13 | S | S | S | S | S | S | S | S | S | S | R | S |
| M14 | R | S | S | S | S | R | S | S | S | S | R | S |
| M15 | S | S | S | S | S | R | S | S | S | S | R | S |
| M16 | R | S | R | S | S | S | S | S | S | S | R | S |
| M17 | R | S | S | S | R | R | S | S | R | S | R | S |
| M18 | S | S | S | S | S | R | S | S | R | S | R | R |
| M19 | R | S | R | S | S | R | S | S | S | S | S | S |
| N1 | R | R | S | S | S | S | S | S | S | S | S | S |
| N2 | R | S | R | S | S | S | S | S | S | S | S | S |
| N3 | R | R | S | S | S | S | S | S | S | S | S | S |
| N4 | S | S | S | S | S | R | S | S | S | S | R | S |
| N5 | S | S | S | S | S | S | S | S | S | S | S | S |
| N6 | R | R | S | S | S | S | S | S | S | S | R | S |
| N7 | S | S | S | S | S | S | S | S | S | S | R | S |
| N8 | S | S | S | S | S | R | S | S | S | S | R | S |
| N9 | S | S | S | S | S | S | S | S | S | S | S | S |
| N10 | S | S | S | S | S | S | S | S | S | S | R | S |
| N11 | S | S | S | S | S | R | S | S | S | S | R | S |
| N12 | R | R | S | S | S | R | S | S | S | R | R | S |
| N13 | S | S | S | S | S | R | S | S | S | S | R | S |
| N14 | S | R | S | S | S | R | S | S | S | S | R | S |
| N15 | S | S | S | S | S | R | S | S | S | S | R | S |
| N16 | R | S | S | S | S | S | S | S | S | S | R | S |
| N17 | S | S | S | S | S | S | R | S | S | S | S | S |
| N18 | R | S | S | S | S | S | S | S | R | S | S | S |
| N19 | S | S | S | S | S | R | S | S | S | S | R | S |
| S N20 | S | S | S | S | S | R | S | S | S | S | S | S |
| N21 | S | S | S | S | S | R | S | S | S | S | R | S |
| N22 | S | S | S | S | S | S | S | S | R | S | S | S |
| N23 | S | S | S | S | S | S | S | S | R | S | R | S |
| N24 | S | S | S | S | S | S | S | S | S | S | R | S |
| N25 | S | S | S | S | S | S | S | S | S | S | S | S |
| N26 | S | S | S | S | S | R | S | S | S | S | R | S |
| N27 | S | R | S | S | S | R | S | S | S | S | R | S |

'S' indicates sensitivity, and 'R' indicates resistance to the tested antibiotics. *PG (Penicillin G, 10 units), E (Erythromycin, 15 µg), AP (Ampicillin, 10 µg), KF (Cephalothin, 30 µg), CD (Clindamycin, 2 µg), TS (Cotrimoxazole, 25 µg), GM (Gentamicin, 10 µg), VA (Vancomycin, 30 µg), FC (Fusidic acid, 10 µg), C (Chloramphenicol, 30 µg), OX (Oxacillin, 1 µg), and CPM (Cefepime, 30 µg).

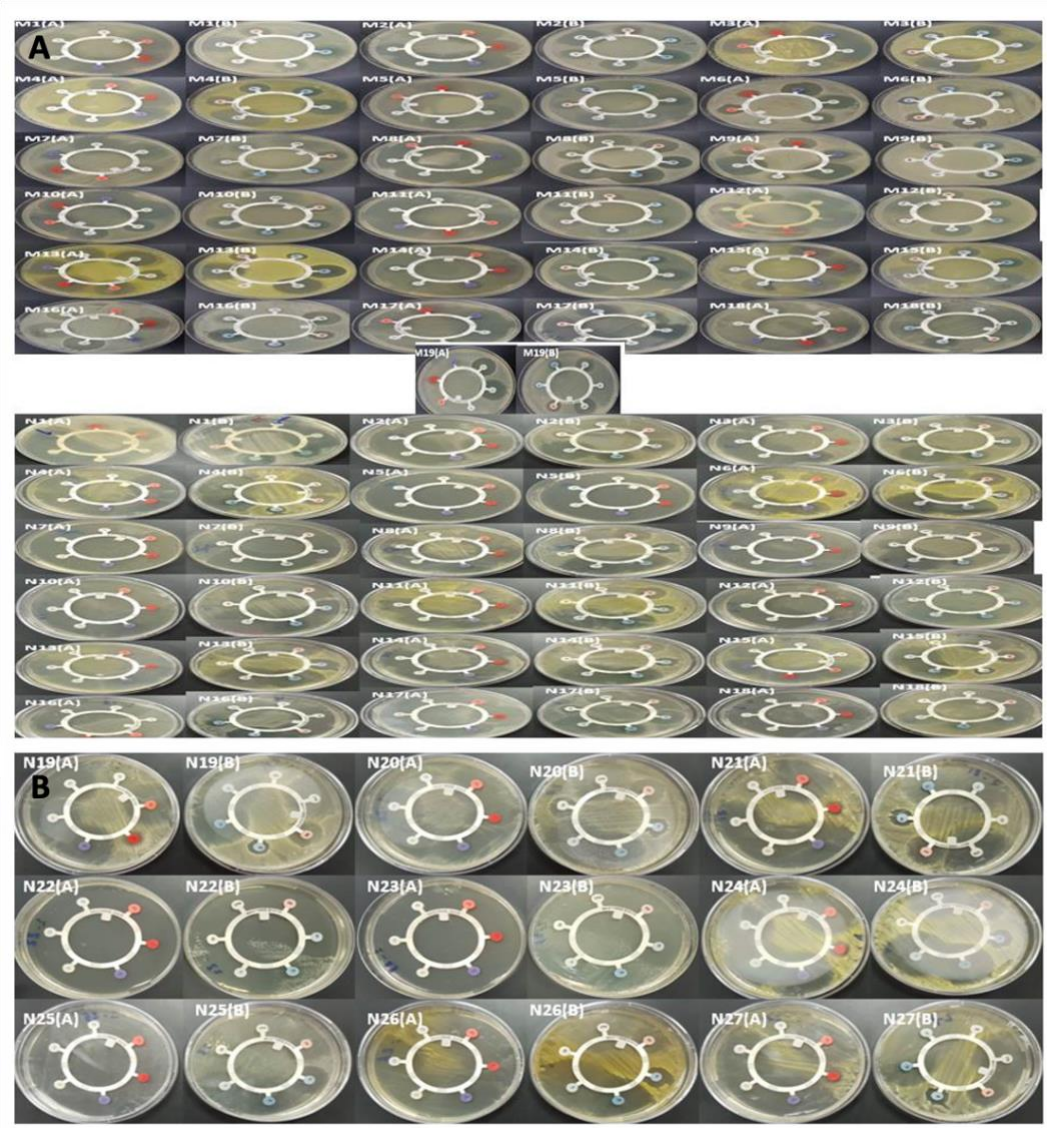


**Figure 1.** Antibiotic susceptibility of the isolated bacteria. A – refers to six antibiotics (PG, E, AP, KF, CD, and TS); B – refers to the other six antibiotics (GM, VA, FC, C, OX, and CPM) tested.

Hemolytic activity of isolated bacteria on blood agar and bacterial growth on MacConkey agar.

The hemolytic activity was observed in the bacteria isolated from both fitness centers. Out of the forty-six (46) bacteria, sixteen (16) bacterial isolates showed strong hemolytic activity (β hemolysis) (Table 3) (Figure 2). Maximum β hemolytic strain activity was exhibited by the isolated from gym 2 (9 strains) followed by gym 1 (7 strains). Only one bacterium (M4) separated from gym 1 showed α hemolytic activity on a blood agar plate, while two (2) strains (N24 and N26) isolated from gym 2 showed a similar hemolytic pattern. However, no hemolytic activity (γ hemolysis) twenty-seven (27) isolates were observed. No bacterial growth was found on the MacConkey agar plate indicating all bacteria were gram-positive (Figure 2).

**Table 3.** The haemolytic activity of bacteria on blood agar plate.

| Bacterial Isolates | Hemolytic pattern |
|---|---|
| M1 | β |
| M2 | γ |
| M3 | γ |
| M4 | α |
| M5 | β |
| M6 | γ |
| M7 | β |
| M8 | β |
| M9 | β |
| M10 | γ |
| M11 | γ |
| M12 | γ |
| M13 | β |
| M14 | γ |
| M15 | γ |
| M16 | γ |
| M17 | γ |
| M18 | γ |
| M19 | β |
| N1 | β |
| N2 | β |
| N3 | γ |
| N4 | γ |
| N5 | γ |
| N6 | γ |
| N7 | γ |
| N8 | γ |
| N9 | γ |
| N10 | γ |
| N11 | γ |
| N12 | γ |
| N13 | β |
| N14 | β |
| N15 | γ |
| N16 | γ |
| N17 | β |
| N18 | β |
| N19 | β |
| N20 | β |
| N21 | β |
| N22 | γ |
| N23 | γ |
| N24 | α |
| N25 | γ |
| N26 | α |
| N27 | γ |

α = greenish discoloration surrounding a bacterial colony growing on the blood agar, β = complete breakdown of red blood cells in the media around and under the colonies, and γ = no hemolysis.

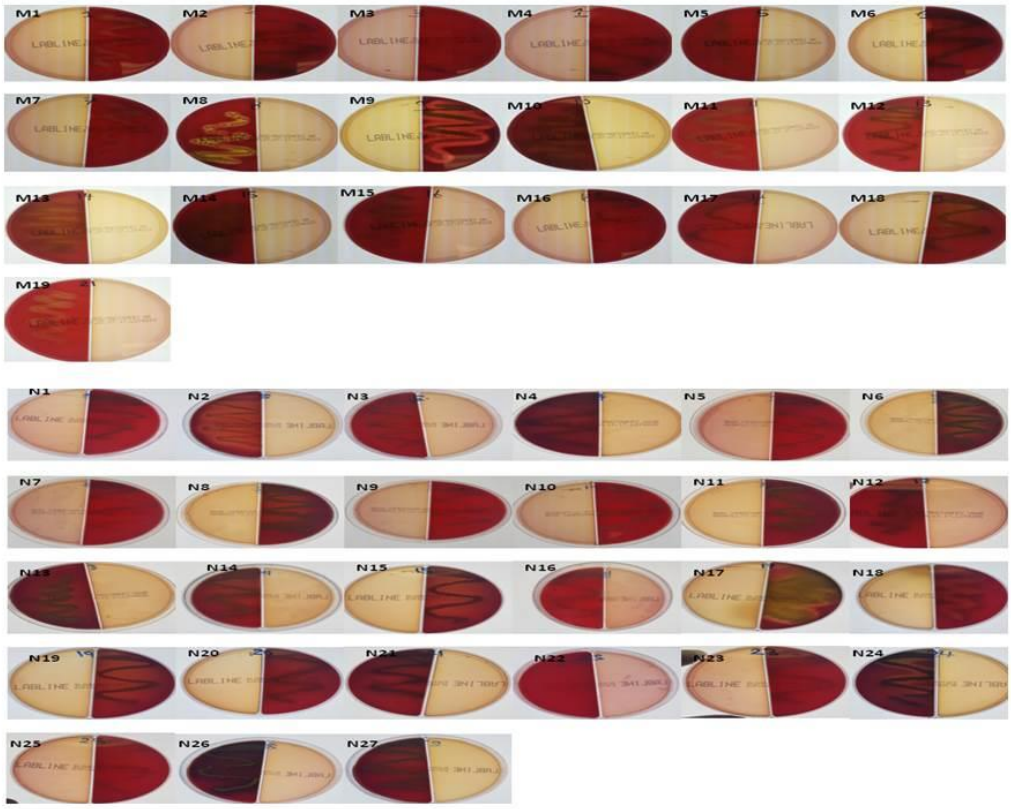

**Figure 2.** The Hemolytic activity of isolated bacteria on blood agar and bacterial growth on MacConkey agar.

**Identification of the isolated bacterial strains**

**Phenotypic and biochemical identification**

Phenotypic characters of all the forty-six (46) isolates were recorded according to the "Bergey's Manual of Systematic Bacteriology" (Table 4). Gram-staining results showed that all bacteria were gram-positive (Table 1). However, out of 46 bacteria, the cell shape of the thirty-four (34) isolates was cocci and of the twelve (12) isolates was bacilli (Table 4). All the isolates were found positive in the catalase test (Table 4).

**Table 4.** Morphological, biochemical, and molecular characterization of the isolated bacteria.

| Bacterial Isolates | Phenotypic features of the isolates | | | | | Biochemical characters of the isolates | | Molecular analysis (based on 16S rDNA sequence) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Colony shape | Colony color | Margin | Surface | Cell shape | Catalase test | Gram reaction | Accession no. of the isolates | Closest species from GenBank | Sequence Similarity (%) |
| M1 | Round | White | Entire | Smooth | Cocci | + | + | MG581160 | *Staphylococcus capitis* | 100% |
| M2 | Round | White | Entire | Smooth | Cocci | + | + | MG581280 | *Staphylococcus epidermidis* | 100% |
| M3 | Round | Lemon yellow | Regular | Smooth | Cocci | + | + | NS | *Brachybacterium nesterenkovii* | 98% |
| M4 | Round | Yellow | Entire | Smooth | Bacilli | + | + | NS | *Microbacterium oryzae* | 97% |
| M5 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus capitis* | 96% |
| M6 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus cohnii* | 99% |
| M7 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus capitis* | 97% |
| M8 | Irregular | White | Lobate | Rough | Bacilli | + | + | NS | *Bacillus subtilis* | 99% |
| M9 | Irregular | Slightly yellowish | Undulate | Smooth | Bacilli | + | + | NS | *Bacillus pumilus* | 99% |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| M10 | Round | Cream | Entire | Smooth | Bacilli | + | + | NS | *Geobacillus stearothermophilus* | 96% |
| M11 | Round | Grayish-white | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus hominis* | 99% |
| M12 | Round | Cream | Entire | Smooth | Bacilli | + | + | NS | *Geobacillus stearothermophilus* | 98% |
| M13 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus aloeverae* | 99% |
| M14 | Circular | Yellow | Entire | Smooth | Bacilli | + | + | NS | *Microbacterium hydrocarbonoxydans* | 97% |
| M15 | Round | Yellow | Entire | Smooth | Bacilli | + | + | NS | *Microbacterium oryzae* | 93% |
| M16 | Round | Yellow | Regular | Smooth | Cocci | + | + | NS | *Staphylococcus pasteuri* | 98% |
| M17 | Round | Cream | Entire | Smooth | Cocci | + | + | NS | Not identified | - |
| M18 | Round | Cream | Entire | Smooth | Bacilli | + | + | NS | Not identified | - |
| M19 | Round | Gray-white | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus warneri* | 98% |
| N1 | Round | Grayish-white | Entire | Smooth | Cocci | + | + | MG581299 | *Staphylococcus hominis* | 100% |
| N2 | Round | Grayish-white | Entire | Smooth | Cocci | + | + | MG581428 | *Staphylococcus hominis* | 99% |
| N3 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus yunnanensis* | 98% |
| N4 | Round | Bright yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus luteus* | 98% |
| N5 | Round | Cream | Entire | Smooth | Bacilli | + | + | NS | Not identified | - |
| N6 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus epidermidis* | 97% |
| N7 | Round | Bright yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus luteus* | 98% |
| N8 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus yunnanensis* | 97% |
| N9 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus epidermidis* | 96% |
| N10 | Round | Bright yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus luteus* | 97% |
| N11 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | Not identified | - |
| N12 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus yunnanensis* | 97% |
| N13 | Round | Bright yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus luteus* | 97% |
| N14 | Irregular | White | Lobate | Rough | Bacilli | + | + | NS | *Bacillus subtilis* | 99% |
| N15 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus epidermidis* | 99% |
| N16 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus yunnanensis* | 98% |
| N17 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus epidermidis* | 99% |
| N18 | Round | Yellow | Entire | Rough | Cocci | + | + | NS | Not identified | - |
| N19 | Round | Bright yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus luteus* | 99% |
| N20 | Round | Bright yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus luteus* | 98% |
| N21 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | Not identified | - |
| N22 | Irregular | Cream | Undulate | Rough | Bacilli | + | + | NS | *Bacillus amyloliquefaciens* | 94% |
| N23 | Irregular | White | Lobate | Rough | Bacilli | + | + | NS | *Bacillus subtilis* | 99% |
| N24 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus yunnanensis* | 99% |
| N25 | Round | Bright yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus luteus* | 98% |
| N26 | Round | Yellow | Entire | Smooth | Cocci | + | + | NS | *Micrococcus yunnanensis* | 98% |
| N27 | Round | White | Entire | Smooth | Cocci | + | + | NS | *Staphylococcus epidermidis* | 99% |

'-' indicates negative response and '+' indicates positive response.; 'NS' refers not submitted yet.

**Molecular Identification of the isolated bacterial strains**

16S rRNA gene sequences of all the 46 bacteria were amplified (Figure 3) and subjected to 16S rRNA gene sequencing (Figure 4) and the results showed that bacterial isolates were mainly the members of six (6) genera, namely, *Bacillus*, *Brachybacterium*, *Geobacillus*, *Microbacterium*, *Micrococcus* and *Staphylococcus* (Table 4). However, maximum numbers of bacteria belonged to the genus *Staphylococcus* (15 isolates) followed by *Micrococcus* (14 strains), *Bacillus* (5 strains), *Microbacterium* (3 strains), and *Geobacillus* (2 strains), respectively. Only one isolate was identified as *Brachybacterium* (M3). However, identification of 6 isolates (M17, M18, N5, N11, N18, and N21) could not be possible based on their 16S rDNA sequences due to very less sequence similarity with available sequences in the GenBank database of NCBI. 16S rDNA sequences of four (4) isolates have been submitted to the GenBank database of NCBI, USA under the accession number MG581160 (strain M1), MG581280 (strain M2), MG581299 (strain N1), and MG581428 (strain N2).

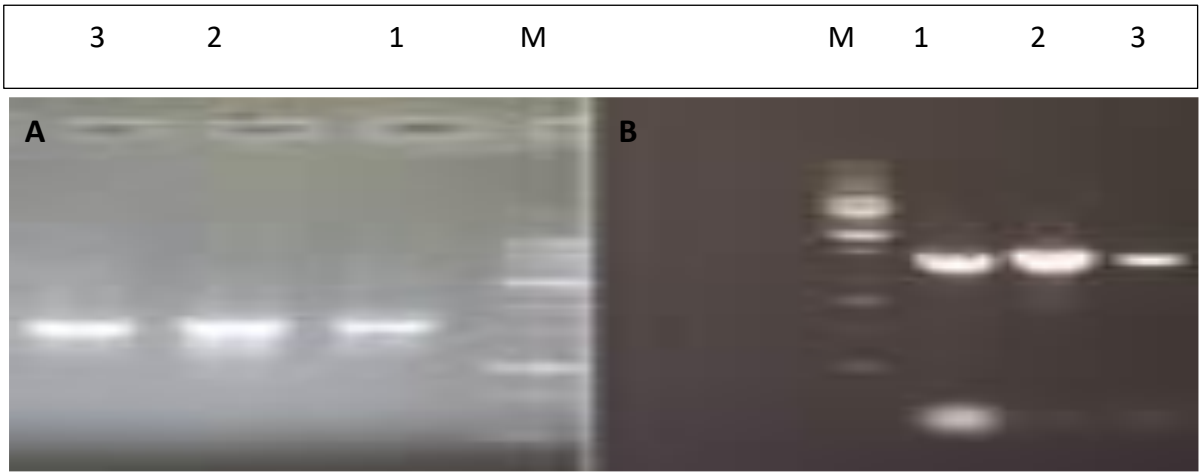


**Figure 3.** 16S rRNA gene PCR products. A – gym 1; B – gym 2.

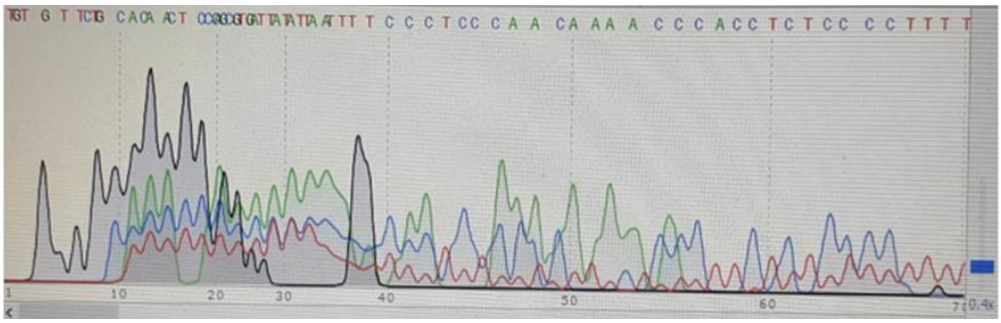

**Figure 4.** 16S rRNA sequences of M1 in SnapGene Viewer software version 3.3.3.

**Phylogenetic analysis**

A phylogenetic tree (Figure 5) was constructed based on the 16S rDNA sequences of the 19 bacteria isolated from gym 1. The tree shows two uncultured bacteria (M17 and M18) are related to *Bacillus pumilus* strain M9. While the 16S ribosomal RNA of three isolates (M4, M14, and M15) is closely related, and they are members of the *Mycobacterium* genus. Although M13 showed sequence homology with *Micrococcus aloe vera*, the phylogenetic tree shows its closeness to *Brachybacterium* (M3). 16S rDNA of isolates M8, M10, and M12 clustered in the same branch and are the members of the Bacillaceae family. All other strains (M1, M2, M5, M6, M7, M11, and M19) isolated from gym 1 showed close relatedness with each other and identified as the members of the genus *Staphylococcus*.

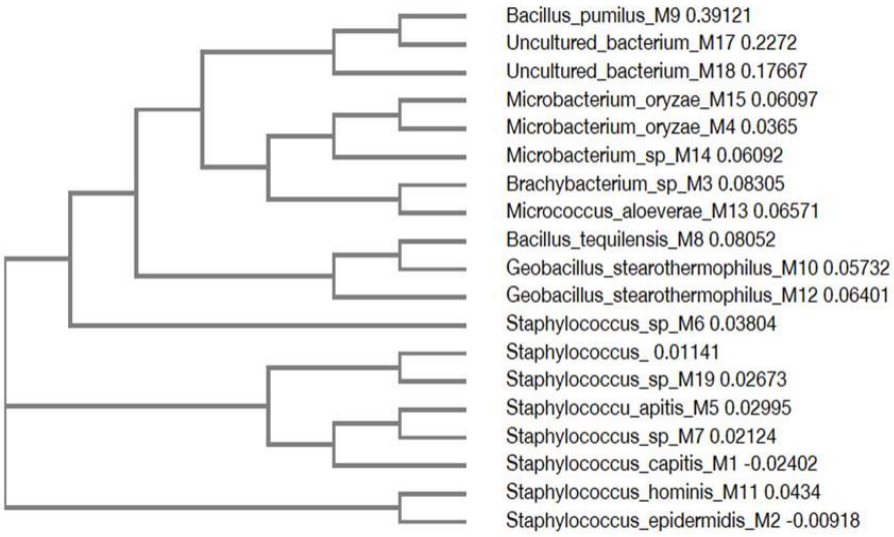


**Figure 5.** Phylogenetic tree based on 16s rRNA sequences of the bacterial isolates from gym 1.

Another phylogenetic tree (Figure 6) based on 16s rRNA of 27 bacteria isolated from gym 2 shows that six strains (N16, N19, N20, N24, N25, and N26) are related to each other and belonging to the members of the genus *Micrococcus*. 11 isolates, namely, N1, N2, N5, N6, N9, N11, N15, N17, N18, N21, and N27 clustered in the same node, where ten isolates are the members of *Staphylococcus* and N5 showed 16s rDNA sequence similarity with the uncultured bacterium. Three strains, i.e., N14, N22, and N23 belong to the genus *Bacillus*. Strains N4, N7, N8, N10, N12, N13 are from the genus *Micrococcus*. However, strain N3 shows closeness in the phylogenetic tree with N7 (*Micrococcus luteus*).

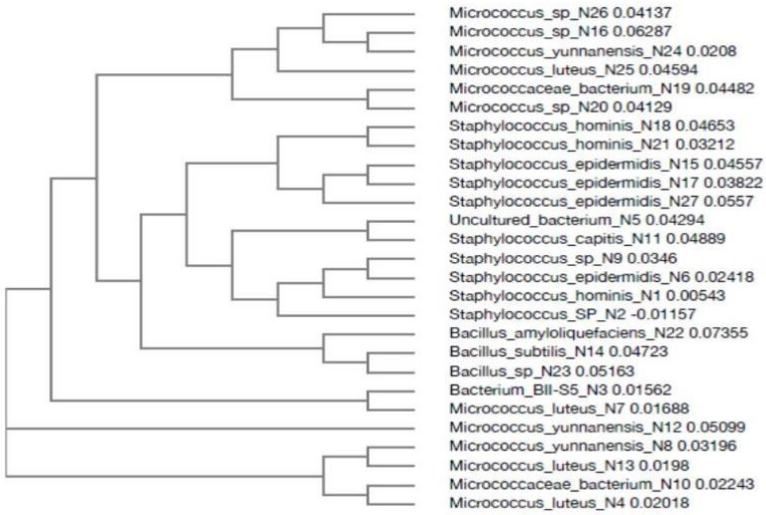


**Figure 6.** Phylogenetic tree based on 16s rRNA sequences of the bacterial isolates from gym 2.

Based on the 16s rDNA sequences of 46 bacteria isolated from gym 1 and gym 2, a phylogenetic tree was constructed (Figure 7). Out of 46 bacteria, 19 strains are closely related to each other in their 16s rRNA gene sequence phylogeny, and they are the members of the genus *Staphylococcus*. However, 15 strains show their 16s rRNA sequence closeness with the genus *Micrococcus*. 7 of the isolated bacteria are related in their 16s rRNA phylogeny, and they belong to the family Bacillaceae includes two genera, namely, *Bacillus*, and *Geobacillus*. However, only a single bacterium (M3) showed maximum sequence similarity with *Brachybacterium,* and it holds a position between the genera *Microbacterium* and *Micrococcus* in the phylogenetic tree (Figure 7).

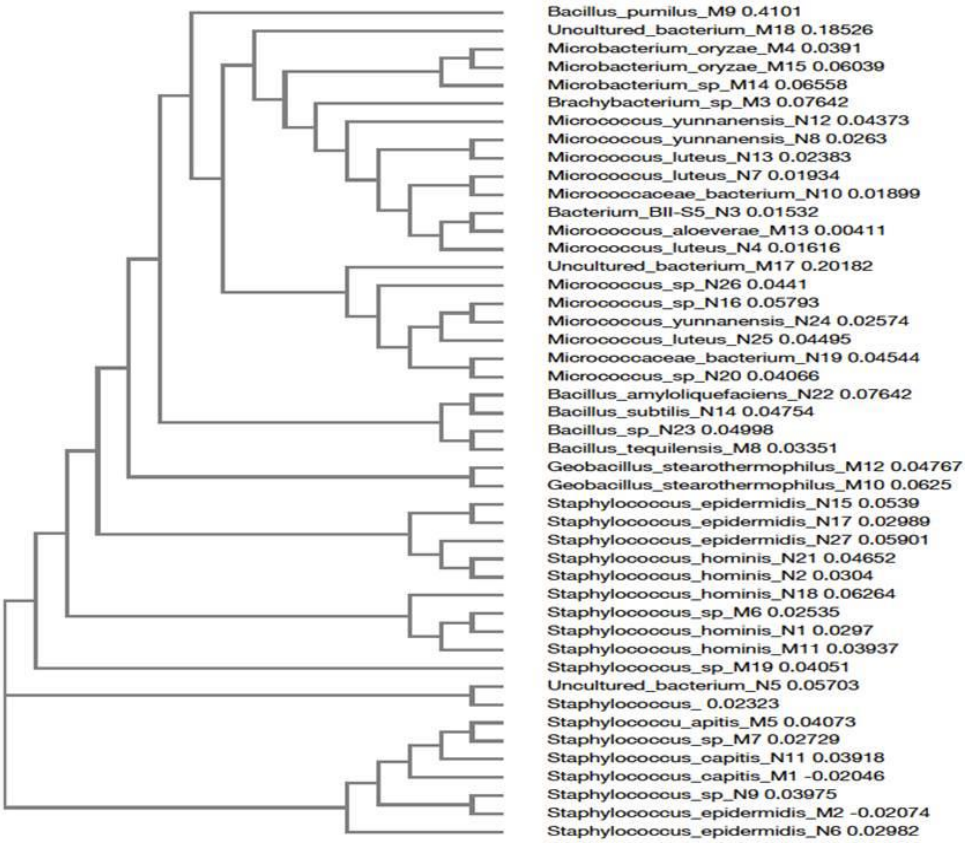


**Figure 7.** Phylogenetic tree based on 16S rDNA sequences of the bacterial isolates from gym and gym 2.

## 4. Discussion

A potential source of pathogenic microorganisms could be fitness centers (Mukherjee et al. 2014). Therefore, the present study was undertaken in two fitness centers located in the Makkah region of Saudi Arabia to understand the overall bacterial population and diversity. A total of forty-six (46) strains of bacteria were isolated from different equipment used in the two chosen gyms.

The antibiotic susceptibility test revealed that 40 bacterial strains had antibiotic resistance to at least 1 of the 12 antibiotics tested among the 46 bacteria. Most significantly, two isolates (M17 and N12) were found to be resistant to the five antibiotics tested. Since its inception, antibiotic resistance has been reported in bacteria. Several studies (Lim et al. 2013; Perron et al. 2015; Ahmed et al. 2020), have reported antibiotic bacterial resistance. The mechanism of bacterial antibiotic resistance was, however, out of the scope of this study, but Blair et al. (2015) reported bacterial ability to be intrinsically resistant to some antibiotics and acquired this characteristic through mutations in chromosomal genes and horizontal gene transfer. To clarify the underlying mechanisms of antibiotic resistance in those strains, further investigation is required. Of the 46 isolated bacteria, β-hemolytic activity on human blood agar plates was shown by 16 strains. However, no hemolytic activity was shown in the maximum strains (27 strains). In many bacterial pathogens, the hemolytic capacity is often regarded as a potent virulence factor, (Rajkumar et al. 2016; Sum et al. 2017). Although the possible reasons for bacterial hemolysis have been reported as distinct hemolysins secreted by bacteria, (Ryan et al. 2009; De Oliveira et al. 2014), the mechanism of this activity is beyond the scope of the present study. Therefore, further research is needed to gain a better understanding of the underlying mechanisms of the active hemolytic strains.

The sequences and subsequent phylogenetic analysis of the 16S rRNA gene sequences of the isolated bacteria showed that they were mainly members of the six genera, namely *Bacillus, Brachybacterium, Geobacillus, Microbacterium, Micrococcus, and Staphylococcus.* Past studies have confirmed that *Staphylococcus and Micrococcus spp.* The bacterial genera found in indoor air environments are the most common, (Górny et al. 2002; Ahmed, et al. 2020). In a study performed in selected fitness centers in a metropolitan area of the USA, Mukherjee et al. (2014) also reported the prevalence of members belonging to the genera Bacillus, Micrococcus, and Staphylococcus, etc. This study, however, was unable to identify six

isolates (M17, M18, N5, N11, N18, and N21). Further biochemical tests and their entire analysis of the genome sequence would be necessary in this regard.

## 5. Conclusion

The present study successfully isolated 46 bacterial strains from various equipment used in the two chosen gyms located in Saudi Arabia's Makkah region. All bacterial isolates are positive for the gram response and catalase test. Some of these strains are resistant to several antibiotics. 16 strains exhibited β-hemolytic activity among the 46. 16S rRNA gene sequencing findings showed that the members of six (6) genera, namely Bacillus, *Brachybacterium, Geobacillus, Microbacterium, Micrococcus, and Staphylococcus,* were primarily isolated bacterial strains. Based on their 16S rRNA sequences, six isolates could not be identified, and therefore, further study is needed to identify these strains. Fitness centers may be a source of potentially pathogenic microorganisms, so regular monitoring of microbial contamination in these exercise centers is essential.

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**Ethics Approval:** Not applicable.

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