

**ANTIBIOGRAM AND MOLECULAR CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM GYM EQUIPMENT IN PUBLIC FITNESS CENTRES IN THOHOYANDOU, VHEMBE DISTRICT, LIMPOPO PROVINCE.**

by

**MASHAU THENDO PRECIOUS (11575281)**

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A Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of Masters of Science in Microbiology (MSc MBY)

to the

**DEPARTMENT OF MICROBIOLOGY  
UNIVERSITY OF VENDA  
PRIVATE BAG X5050  
THOHOYANDOU  
0950**

**SUPERVISOR: DR E.M. MUSIE**

**CO-SUPERVISOR: DR T. MASEBE**

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## SUMMARY

**Introduction:** *Staphylococcus aureus* (*S. aureus*) has emerged as an important cause of skin and soft tissue infections. It is a major pathogen of increasing importance due to the rise in antibiotic resistance. Exercise equipment in public fitness centres are used repeatedly by multiple people every day and this exposes the equipment to different forms of microbial contamination and also exposes equipment users to different opportunistic infections/pathogens. Transmission of *S. aureus* is known to occur by hand and body contact with fomites. Exercise equipment, may serve as fomites for the transmission of *S. aureus* in public fitness centers. To date, there is little evidence that gym surfaces serve as reservoirs for *S. aureus*. The main objective of the study is to determine the prevalence and characteristics of *S. aureus* from exercise equipment in public fitness centres in Thohoyandou, Vhembe district, Limpopo province.

**Materials and Methods:** Five hundred Samples were collected for a period of 5 months (May to September) from three local fitness centers (private community gymnasium, university student gym and university biokinetics gymnasium) in Thohoyandou. Hand contact surfaces of often used exercise equipment were sampled using sterile swabs and saline solution. Exercise equipment were swabbed for 10 seconds and the swab placed into Amie's Transport Media and then transported on ice (4 °C) to the Microbiology Laboratory at University of Venda, for further analysis.

Swabs were inoculated into Baird Staphylococcus enrichment broth and incubated at 37°C for 24 hours. The inoculum was then streaked onto Mannitol Salt Agar (MSA) and plates were incubated at 37°C for 48 hours. Yellow colonies with yellow zones produced were presumed to be *S. aureus* strains. *S. aureus* isolates were further identified using morphological Gram staining test and biochemical tests such as Catalase and *Staphylococcus* latex agglutination test. Confirmed *S. aureus* isolates were subjected to antibiotic susceptibility test in order to determine their susceptibility to antibiotics using Kirby-Bauer Disc Diffusion method. A sterile swab was dipped into the 0.5 McFarland culture suspensions and streaked onto Mueller-Hinton agar plate for a lawn of growth. Antibiotic disks impregnated with compounds to be tested were placed on the surface of the agar using a dispenser. The plates were inverted and incubated for 24 hours at 37°C. A diameter of the zone of inhibition was measured. MIC and antibiotic susceptibility of isolates were determined using E-test strips. The breakpoints used to define susceptible, resistant, and intermediate categories for each antimicrobial agent were confirmed according to the CLSI (Clinical Laboratory Standard Institutes) document M100-S22, 2012. Detection and

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amplification of *nuc*, *spa*, *mecA* and *pvl* were performed using a multiplex PCR. A total of 10 amplified samples were sequenced using Dye-terminator Sanger sequencing to confirm the presence of the *mec*, *nuc* and *pvl* gene. The resulting sequences were manually edited with the SeqMan pro Version 8.1 in the DNASTAR software (DNASTAR, INC, Madison, Wisconsin, USA) and nucleic acid and the predicted amino acids were aligned with respective *S. aureus* reference strains obtained from GenBank using Clustal W multiple aligner.

**Results:** One hundred and eighty one (36%) of the 500 samples collected from 7 different exercise equipment were found to be colonized with *S. aureus*. The most colonised gym was student gym (43%) followed by private community gym (34%) and university bio kinetics (26%). The most colonised equipment was Benches (48%) followed by Barbells (44%), Upright bike (40%), Treadmill (35%) and Spinning bike (28%). Ninety (50%) *S. aureus* isolates were resistant to both cefoxitin and oxacillin (MRSA). The isolates were also resistant to ampicillin (81%) and highly susceptible to vancomycin (98%), cefazolin (92%) and gentamycin (86%). The mean MICs for Ampicillin, Cefoxitin, Oxacillin, Amoxicillin, Gentamicin and Vancomycin were 1, 3.83, 1.7, 1.2, 0.5 and 2 µg/ml, respectively.

Out of 90 MRSA isolates tested 37(41%) were found to have the *S. aureus* specific gene (*nuc* gene), 8(26%) had *mecA* gene, *spa* protein gene was detected in 21(57%) isolates and 2(2%) isolates were found to possess *pvl* virulence gene. When aligned with the *S. aureus* reference strains, the study sequences (*nuc*, *mecA*, *pvl*) showed 98.9-100% similarities to the reference genes (strains).

**Conclusion:** This study has demonstrated the presence of MRSA and MSSA in gymnasium settings in Thohoyandou. It was observed that *S. aureus* strains possessed methicillin resistance gene (*mecA*) and virulence genes (*pvl* and *spa* genes). Therefore, gymnasiums may be a significant source of pathogenic *S. aureus* infections. This study has shown that the combination of susceptibility testing and molecular methods provides useful information on the antibiotic resistance and characteristics of *S. aureus*. The high proportion of MRSA (PVL positive) observed in this study indicates that adequate measures are needed in order to prevent and control the spread of *S. aureus* in public fitness centres. MRSA may be difficult to treat once infection has taken place. Further studies to determine the source of MRSA and the extent to which MRSA contamination contributes to skin and soft tissue infections would be recommended.

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