Antimicrobial Susceptibility of Staphylococcus Aureus Isolated from Recreational and Natural Water Bodies in Lusaka, Zambia

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Competing Interests
The authors declare that they have no competing interests.

DOI: *https://10.53974/unza.jabs.5.3.751*

Abstract

Introduction: *Staphylococcus aureus* is a potentially harmful human pathogen associated with nosocomial and community-acquired infections with increasing antibiotic resistance. Although microbial contamination of marine waters is predicted to be responsible for millions of gastrointestinal and acute respiratory infections and several skin infections, there is little information regarding the microbial contamination of water bodies in many sub-Saharan countries. Therefore, this study aimed at determining the antimicrobial susceptibility of *S. aureus* isolated from recreational waters and natural water bodies in Lusaka, Zambia.

Methods: This was a cross-sectional study with a total of 90 water samples collected from recreational and natural water bodies. To isolate *S. aureus,* standard microbiological methods were used, while the Kirby-Bauer disk diffusion method was used for susceptibility testing. Methicillin-resistant *Staphylococcus aureus* was detected by the use of cefoxitin.

Results: The overall results showed that there was 36.7% bacterial contamination in the waters tested. From the 90 samples collected, a total of 33 bacteria were isolated, of which 12 (36.4%) were *Coagulase Negative Staphylococcus*, 9 (27.2%) were *S. aureus,* and 12 (36.4%) were *non-staphylococcus species.* All the isolates showed a 100% resistance to penicillin G and ampicillin. The *S. aureus* isolates were most susceptible to chloramphenicol (88.9%), cefoxitin (88.9%), ciprofloxacin (100%), amikacin (88.9%) and gentamicin (88.9%). Only 11.1% of isolates showed phenotypic resistance to methicillin after testing against cefoxitin.

Conclusion: The results from this study signify that recreational and natural water bodies in Lusaka, Zambia, may be possible reservoirs of antibiotic-resistant *S. aureus,* which may possibly be transmitted to humans when using the same waters.

Keywords: Antimicrobial Resistance, Recreation, *Staphylococcus aureus,* Water Bodies, Zambia

Introduction

Staphylococcus aureus is a potentially harmful human pathogen associated with nosocomial and community-acquired infections [1]. Yearly, microbial contamination of marine waters is predicted to be responsible for millions of gastrointestinal and acute respiratory infections and several skin infections [2]. *S. aureus* is a recognised health hazard in swimming pools and beaches, where it is assumed to be derived mainly from bather shedding. That is, swimmers' dissemination of *S. aureus* in marine water, via the shedding of the bacterium from their nose, skin, and respiratory tract [2]. The human skin is directly exposed to infectious agents during swimming [2]. Hence, the environment plays an important role in the transmission of microbial agents to humans [3]. In addition, the most common media of microbial transmission are water, soil, air and certain objects such as door handles, among others [4].

Furthermore, antimicrobial resistance (AMR) amongst bacteria is a growing problem worldwide [5]. The overuse of antimicrobials elicits resistance either by the emergence of point mutations or by the acquisition of foreign resistance genes, which leads to alteration of the antimicrobial target and the degradation of the antimicrobial or reduction of the cell's internal antimicrobial concentration [2]. However, *S. aureus* is well known to harbour resistance to several antimicrobial agents such as methicillin, ampicillin, penicillin, rifampicin, clindamycin, and erythromycin, among others, which may lead to treatment challenges and costs related to the more extended hospital stay. The emergence and global spread of multi-drug resistant strains, particularly methicillin-resistant *S. aureus* (MRSA) strains, is worrisome as they are practically resistant to many antibiotics.

Zambia has not been spared from the increase in the number of antibiotic resistance cases due to *S. aureus*. For instance, in recent years, nosocomial infections due to MRSA have been reported to be on the increase at the University Teaching Hospital [6]. While studies from around the world have shown that *S. aureus* and MRSA have been isolated from different marine water, stream water, intertidal sand samples and beaches [2, 7-9], there is a paucity of data on the presence of these organisms in the recreational and natural water bodies in many sub-Saharan countries including Zambia. Therefore, this study aimed at determining the antimicrobial susceptibility of *S. aureus* isolated from recreational and natural water bodies in Lusaka, Zambia.

Materials and Methods

Study Design and Site

This was a descriptive cross-sectional study conducted in selected areas of Lusaka, the capital city of Zambia. Lusaka is the largest city in the country, with an estimated urban population of about 2.5 million, according to the Central Statistics Office (CSO) of Zambia in the year 2019. The city has several recreational and natural water bodies where people go to have some recreational activities. It is also a centre for trading and the most visited city by international and local visitors.

3.3 Study Frame and Sample Size

The study included recreational and natural water bodies mostly visited and used in Lusaka, including four recreational swimming pools at a hotel, a lodge, a recreational club and amusement park, three streams, and two water dams. A total of 90 water samples were collected (10 samples per water body).

Sample Collection from Pools and Natural Water Bodies

Samples were collected in the morning, at noon and in the evening by collecting 250ml of water in sterile containers and taken to the laboratory for culturing within two to three hours of collection. All water samples were cultured within 24 hours upon collection or refrigerated at 4ºC if not cultured within this stipulated time until cultured.

Detection of *S. aureus*

After collection, the samples were inoculated on Mannitol salt agar (Oxoid, UK Ltd) plates and were incubated at 37°C for 24 to 48 hours. After 24 to 48 hours of incubation, the plates were examined for bacterial growth. The plates with bacterial growth were further examined macroscopically for obvious growth characteristics like colour, texture, shape and size that could be useful in analysing the specimen content. The Gram stain was done to group the microorganisms into Gram-positive and Gram-negative. After microorganisms were grouped into Gram-positive and negative, biochemical identification of the bacteria was carried out. For Gram-positive cocci isolates, a catalase test was used to differentiate *Staphylococcus* species from *Streptococcus* species. Then a coagulase test was used to distinguish *S. aureus* from the other *Staphylococci.*

Determination of the Antimicrobial Susceptibility Patterns of the *S. aureus*

Isolates confirmed by biochemical test as *S. aureus* were subjected to antimicrobial susceptibility testing against penicillin G (10units), ampicillin (10µg), gentamycin (10µg), erythromycin (15µg), ciprofloxacin (5µg), tetracycline (30µg), amikacin (30µg), clindamycin (2µg), and chloramphenicol (30µg) (Oxoid, UK Ltd) using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK Ltd) according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. The plates were incubated at 37°C for 24 hours, and quality control was performed by using a reference strain of *S. aureus* ATCC 25923, and then diameters of zone of inhibition were measured, and results were interpreted as susceptible (S), intermediate (I) and resistant (R) according to CLSI [10].

Detection of Methicillin-Resistant *Staphylococcus aureus*

All isolates confirmed to be *S. aureus* by biochemical tests were subjected to antibiotic susceptibility testing to cefoxitin (30µg) (Oxoid, UK Ltd) by disc diffusion test to determine phenotypic resistance to methicillin. The inoculated plates were incubated at 35ºC for 24 hours. All isolates that showed resistance to cefoxitin were considered to be presumptive MRSA.

Data Analysis

Data obtained from this study were analysed using the GraphPad Prism Software Version 6.0 for Windows. Descriptive data such as proportions of isolates and their susceptibility patterns were analysed and presented in graphs.

Ethics Considerations

Ethical approval was obtained from the University of Zambia Health Science Research Ethics Committee, approval number 20190217056. Permission to collect water samples from pools was obtained from the relevant authorities of the hotel or lodge, and permission to collect water samples from natural water bodies was obtained from the Lusaka City Council and other relevant authorities. Confidentiality was maintained by anonymising the data.

Results

Detection of *S. aureus* from Waters

The overall results showed that there was 36.7% bacterial contamination in the waters tested. From the 90 samples collected, a total of 33 bacteria were isolated, of which 12 (36.4%) were *coagulase-negative Staphylococcus* (CoNS), 9 (27.2%) were *Staphylococcus aureus,* and 12 (36.4%) were *non-staphylococcus species* as shown in Figure 1. The natural water bodies yielded more bacterial isolates than the swimming pools (Figure 2).

Antimicrobial Susceptibility Patterns of the *S. aureus*

Most of the isolates were generally very susceptible to many of the antibiotics tested in the study, including ciprofloxacin 100% (9/9), chloramphenicol 88.9% (8/9), amikacin 88.9% (8/9) and gentamicin 88.9% (8/9). Lower susceptibility was recorded for tetracycline and clindamycin at 55.6% (5/9) and erythromycin at 66.7% (6/9). All the *S. aureus* isolates showed 100% resistance to penicillin G and ampicillin. Resistance to erythromycin was 22.2% (2/9), clindamycin and tetracycline recorded 44.4%, chloramphenicol, gentamycin and amikacin each recorded 11.1%. The percentage of antimicrobial resistance of *S. aureus* isolates is shown in Figure 3.

Phenotypic Detection of MRSA

Only 11.1% (1/9) of the *S. aureus* isolates showed phenotypic resistance to methicillin by cefoxitin susceptibility, while the rest, 88.9% (8/9), were susceptible.

Discussion

Over the past decade, the percentage of *S. aureus* isolated from different water bodies has shown a slight increase in resistance to a range of antimicrobial agents worldwide [7-9, 11]. The present study sought to detect the presence of *S. aureus* and other *Staphylococci* from different recreational and natural water bodies within the Lusaka District of Zambia and determine the antimicrobial susceptibility of *S. aureus* isolates. Overall, bacterial contamination was detected in 36.7% of the water bodies sampled in the study. The organisms which were isolated from the water include *CoNS, S. aureus* and non-*Staphylococcus* species.

This study revealed a prominent CoNS species (36.4%) presence in all the bacterial isolates. These findings were in agreement with the study done in Spain, which demonstrated a higher presence of CoNS [8]. Although CoNS have a benign interaction with the host, these species are now known to cause critical infections, especially in immunocompromised patients and have gained increasing importance in the healthcare field [12, 13]. Generally, CoNS produces several clinical syndromes, including indwelling devices' infections, post-surgical endophthalmitis, prosthetic valve endocarditis, osteomyelitis, urinary tract infections, and bacteraemia in immune-compromised hosts. The high rate of CoNS in this study might be due to the fact that these bacteria represent a regular part of the microbiota of the skin and mucous membranes of humans. Hence, they are expected to be present in water bodies once people swim in them. Furthermore, CoNS can survive in water for an extended period of time since they prefer higher humidity areas [14].

This study showed that the incidence of isolation of *S. aureus* was 27.2%. Comparable results were reported in Nigeria [11], Spain [8], and Northeast Ohio, in the United States of America (USA) [9], whose isolation rates ranged from 22% to 30%. However, the incidence of *S. aureus* isolation in this study was lower than in a study done by Goodwin and McNay [15] in California, USA, whose incidence rate was 59%. This difference might be due to the smaller sample size of our study and the low turn up of the people using the swimming pool at the time of data collection, which was done in the cold season. Additionally, differences in sample processing methods and geographical location could contribute to the differences in the findings of this study.

Further, this study demonstrated variations in contamination between the natural water bodies (88%) and swimming pools (12%). The fact that the highest contamination rate was from the natural water bodies is quite disturbing in the sense that these places are easily accessed with minimal restriction. Thus, a high risk of transmission of infections to the people who are exposed. The low detection in the pools might be due to effective and efficient disinfection of pools by the owners and fewer people using the facilities, since sampling was done in the cold season.

Antibiotic susceptibility of the isolates showed varying degrees of susceptibility patterns against the antimicrobial agents, of which all the isolates showed 100% resistance to penicillin G and ampicillin. Our findings correlate with the findings of Akanbi, Njom [2]. The high resistance to these Beta-lactam antibiotics was expected, as they have been the most commonly used antibiotics for the treatment of infections in humans and animals since 1961, with penicillin developing resistance to *S. aureus* since the 1960s [16]. However, what is worrying is that ampicillin-resistant isolates may cross select for resistance to other beta-lactams hence an indication of the resistance of the isolates to other beta-lactam antibiotics [2]. In this study, chloramphenicol, ciprofloxacin, amikacin and gentamicin were the most effective drugs against *S. aureus.* In this study, *S. aureus* showed varying resistance to the following drugs: erythromycin (22.2%), chloramphenicol, gentamycin, and amikacin, each recorded 11.1%, tetracycline and clindamycin recorded 44.4%. These results were in agreement with the study done by Thapaliya and Hellwig [9] in the USA, although the present study showed an increase in resistance to tetracycline and clindamycin. These results are very important as they indicate an unusual increase in the resistant strain of *S. aureus*, which could be a public concern in the near future. Additionally, the emergence of drug-resistant strains of *S. aureus* may result in re-infection, prolonging the hospital stay.

The prevalence of cefoxitin-resistant *S. aureus* was 11.1%, and these results agreed with the study done in the USA [9]. However, an 11.1% cefoxitin resistance was of concern despite being low due to the fact that a small number of isolates was evaluated. In addition, resistance to cefoxitin is currently taken as the surrogate marker for resistance due to the *mec*A gene [10]. Furthermore, all *S. aureus* isolates evaluated in this study were multidrug-resistant. This is of significance as it potentially could lead to failure in treatment, prolonged illnesses, increased expenses for healthcare, and in severe cases, and risk of death if humans are infected with such strains. The finding of this study is important concerning humans since the water bodies studied are used by people and could act as a reservoir for the transfer of microbes from one person to another [1]. Hence, these water bodies could play an important role in the transmission of microbial agents in the community and thereby pose a major public health concern [1]

The study is not without limitations. A relatively small sample size was used. Only conventional methods were used to detect the bacteria, and speciation was not thoroughly achieved. Therefore, more studies should be conducted around the country to obtain insight into this matter to determine the extent of the problem and identify all bacteria that may be detected in the waters. Additionally, molecular analyses of the bacteria to determine the antimicrobial resistance genes are recommended.

Conclusion

This study showed that swimming pools and dams might be potential reservoirs for transmission of antibiotic-resistant *S. aureus* and other *Staphylococci* to people who usually go and swim into these waters, particularly, those with skin lesions. A total of 33 bacteria were isolated from these waters, of which 12 (36.4%) were CoNS, 9 (27.2%) were *S. aureus,* and 12 (36.4%) were *non-staphylococcus species.* Antibiotic susceptibility of the isolates showed varying degrees of susceptibility patterns against the antimicrobial agents, of which all the isolates showed 100% resistance to penicillin G and ampicillin. Chloramphenicol, ciprofloxacin, amikacin and gentamicin were the most effective drugs against *S. aureus,* and 11.1% of isolates showed phenotypic resistance to methicillin after testing against cefoxitin. Therefore, the results from this study signify that recreational waters and natural water bodies in Lusaka, Zambia, may be possible reservoirs of antibiotic-resistant *S. aureus*, which may possibly be transmitted to humans when using the same waters.

Acknowledgements

The authors would like to thank the management of the swimming pools and local authorities for the permission granted to collect the water samples. They also thank the management at the University Teaching Hospital, Lusaka, Zambia, for allowing them to conduct the experiments at the hospital laboratory. Special thanks also go to the laboratory technicians who assisted in the data collection process.

Authors' Contributions

Lackson Mwape and Annie Kalonda conceived the idea; Lackson Mwape, Kaunda Yamba and Mulemba Tillika Samutela collected the data; Lackson Mwape and Annie Kalonda analysed the data; Kaunda Yambaprovided reagents; Mulemba Tillika Samutela drafted the manuscript; Lackson Mwape, Kaunda Yamba, Mulemba Tillika Samutela, and Annie Kalonda critically reviewed the manuscript. All authors read and approved the final draft of the manuscript. Annie Kalonda and Mulemba Tillika Samutela supervised the research.

Competing Interests

The authors declare that they have no competing interests.

Funding

This research did not receive any special funding.

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Figures

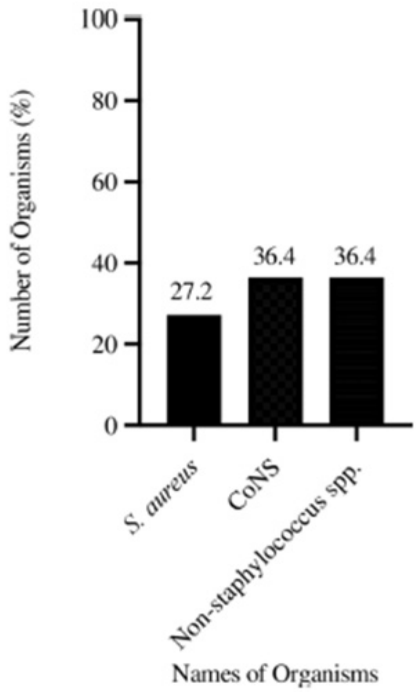

Figure 1: **Overall frequency of bacterial isolates from different water bodies. S. aureus – Staphylococcus aureus and CoNS – Coagulase-negative Staphylococcus**.

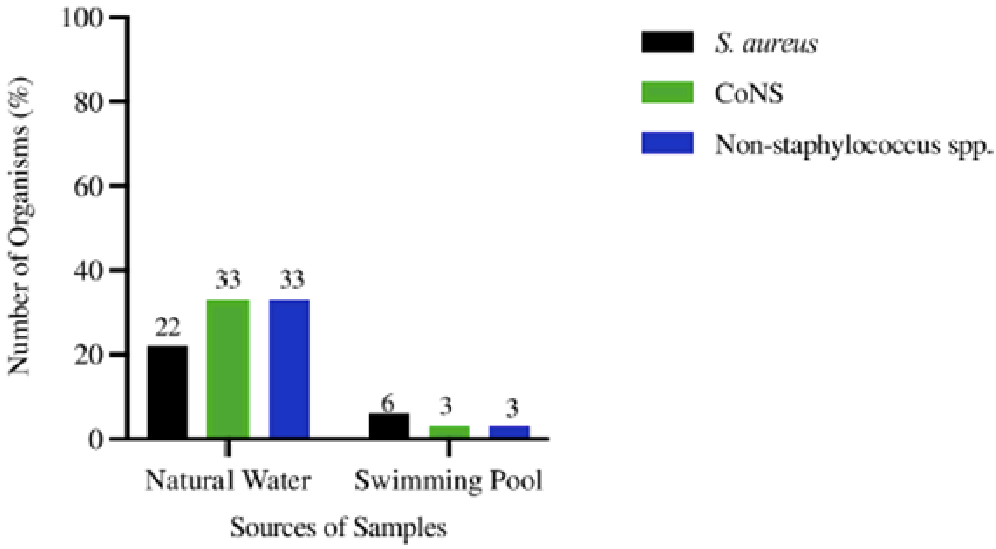

Figure 2: The distribution of organisms in water bodies across the nine sampling sites (3/9 natural water and 6/9 swimming pools)

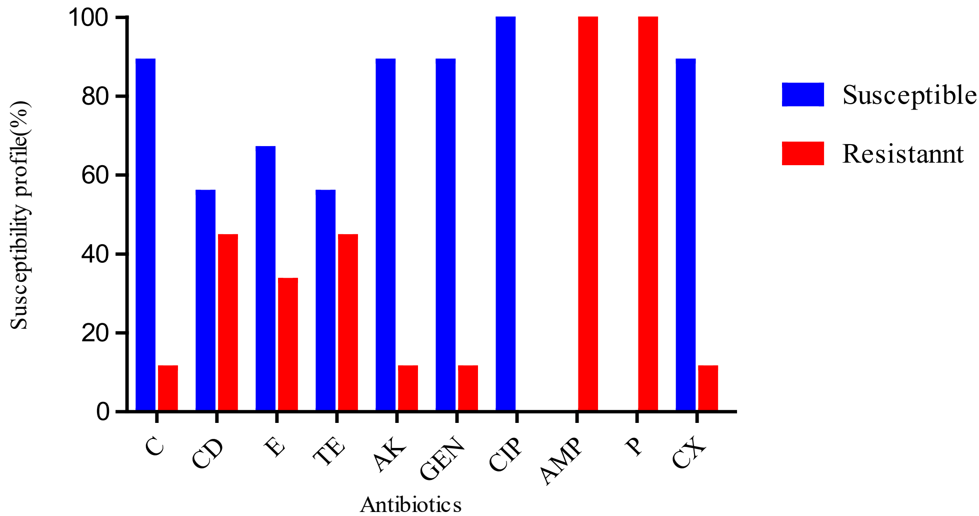

Figure 3: Antimicrobial Resistance Profiles of S. aureus Isolates. C-chloramphenicol; CD-clindamycin; E-erythromycin; TE-tetracycline; AK-amikacin; GEN-gentamicin; CIP-ciprofloxacin; AMP-ampicillin; P-penicillin; CX-cefoxitin.