



Antimicrobial Susceptibility Profiles of Methicillin-Resistant *Staphylococcus aureus* isolates from the University Teaching Hospital, Lusaka, Zambia

Mulemba T. Samutela^{1*}, James C.L. Mwansa², Annie Kalonda¹, Enoch M. Mumbula³, Trevor Kaile³, Clemence Marimo³, Lydia Korolyova³, Bernard Mudenda Hang'ombe⁴, Edgar Simulundu⁵, Chileshe Lukwesa-Musyani², Geoffrey Kwenda¹

¹Department of Biomedical Sciences, School of Medicine, University of Zambia, Lusaka, Zambia

²Department of Pathology and Microbiology, University Teaching Hospital, Lusaka, Zambia

³Department of Pathology and Microbiology, School of Medicine, University of Zambia, Lusaka, Zambia

⁴Department of Para-clinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia

⁵Department of Infectious Diseases, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia

Abstract

Staphylococcus aureus is implicated in nosocomial infections worldwide and is associated with a variety of infections such as endocarditis, food poisoning, toxic shock syndrome, septicaemia, skin and soft tissue infections, and bone infections. The emergence and spread of multi-drug resistant strains of *S. aureus*, particularly methicillin-resistant strains, is worrisome as they are resistant to many antibiotics. However, there is sparse information on the burden of methicillin-resistant *Staphylococcus aureus* in Zambia. Knowledge of antimicrobial susceptibility patterns of bacterial pathogens is crucial for optimal treatment of patients. It is also important for monitoring the spread of resistant organisms in hospitals and communities. The objective of this study was to identify and determine the antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* at the University Teaching Hospital in Lusaka, Zambia. A total of 95 stored isolates of suspected methicillin-resistant *Staphylococcus aureus* from pus and blood specimens collected from June 2009 to December 2012 at the University Teaching Hospital were analysed. Conventional microbiological methods and Clinical Laboratory Standards Institute criteria were used to identify and determine the antimicrobial susceptibility of isolates. Of the 95 *S. aureus* isolates, 43% were identified as methicillin-resistant *Staphylococcus aureus* strains. These methicillin-resistant *Staphylococcus aureus* strains were resistant to trimethoprim/sulfamethoxazole (100%), ciprofloxacin (95%), penicillin (95%), erythromycin (79%), tetracycline (76%) and gentamicin (67%). Multi-drug resistance to a combination of, four, five, six and seven antibiotics was observed in 17.5%, 27.5%, 35%, and 17.5% of the methicillin resistant *Staphylococcus aureus* isolates, respectively. The prevalence of multi-drug resistant methicillin resistant *Staphylococcus aureus* at the University Teaching Hospital was found to be high. Regular surveillance for multi-drug infections is recommended for infection control and to guide treatment.

Key Words: MRSA, *Staphylococcus aureus*, Multi-drug resistance, Antimicrobial susceptibility

*Corresponding Author: Ms Mulemba Tillika Samutela, Department of Biomedical Sciences, University of Zambia, School of Medicine, P.O. Box 50110, Lusaka. Email: mulembats@gmail.com

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Introduction

Staphylococcus aureus is one of the major causes of nosocomial infections worldwide [1, 2]. It is associated with a variety of infections such as endocarditis, food poisoning, toxic shock syndrome, septicaemia, skin and soft tissue infections, and bone infections [3]. Furthermore, *S. aureus* is well known to harbour resistance to a number of antimicrobial agents, which may lead to treatment challenges and the cost related to longer hospital stay [4-6]. 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 [4, 7, 8].

Resistance to methicillin in *S. aureus* is primarily due to the expression of penicillin-binding protein 2a (PBP2a/ PBP2) with reduced affinity for β -lactam antibiotics [9, 10]. These PBPs are encoded by the *mecA* gene, and more recently the *mecC* gene, which are carried on the mobile staphylococcal chromosomal cassette (SCC*mec*) [11]. Several SCC*mec* types exist, and these may also encode resistance genes to other antibiotics [10, 12-15]. In addition to hospital-acquired MRSA (HA-MRSA), community-acquired MRSA (CA-MRSA) has increasingly been found to cause severe disease [10, 16-19]. MRSA has also been reported in livestock (LA-MRSA) [20-22].

There is a paucity of data on MRSA in most African countries, and in most of these countries the prevalence of MRSA has been progressively increasing [23-30]. In Zambia, previous studies carried out at the University Teaching Hospital in Lusaka have shown that the burden of MRSA is also growing, indicating an increase from 23% in 2003 to 37% in 2012 [31-33]. The objective of this study was to identify and determine the antimicrobial susceptibility patterns of MRSA at the University Teaching Hospital, a tertiary reference hospital in Lusaka, Zambia.

Materials and Methods

Bacterial Isolates: Ninety five suspected single clinical MRSA isolates obtained from pus and blood specimens from June 2009 to December 2012 at the University Teaching Hospital in Lusaka, Zambia, as part of the routine hospital care were analysed in this study.

Culture Conditions and Media: The clinical isolates were first plated onto Columbia blood agar plates (Mast Group Ltd, Merseyside, UK) and incubated at 37°C for 24 hours. *S. aureus* isolates were identified by standard microbiological methods including colony morphology, Gram stain, catalase reaction, coagulase activity and DNase test [34].

Antimicrobial Susceptibility Testing

Methicillin Resistance Detection: Screening for resistance to methicillin was performed using 1 μ g oxacillin and 30 μ g cefoxitin disks using the Kirby-Bauer disc diffusion method on Muller-Hilton agar media (Mast Group Ltd, Merseyside, UK) without sodium chloride supplementation according to the

Clinical and Laboratory Standards Institute guidelines [35]. The confirmation of MRSA was based on PCR detection of the *mecA* gene using a previously described multiplex PCR protocol [36, 37].

Antimicrobial Susceptibility Testing to Other Drugs: Antibiotic susceptibility to several anti-staphylococcal antibiotics was performed by the Kirby-Bauer disc diffusion method according to CLSI guidelines: 10 units penicillin G, 15 μ g erythromycin, 2 μ g clindamycin, 25 μ g trimethoprim/sulfamethoxazole, 30 μ g vancomycin, 30 μ g teicoplanin, 30 μ g chloramphenicol, 5 μ g ciprofloxacin, 10 μ g gentamicin, 30 μ g amikacin, and 30 μ g tetracycline (Mast Diagnostics Ltd, Merseyside, UK). Inducible macrolide-streptogramin (MLS_{Bi}) was detected using the double erythromycin-clindamycin disc test (D-test). *S. aureus* strain ATCC 25923 was used as the control strain. Isolates were classified as multi-drug resistant (MDR) if, in addition to the β -lactams, they were resistant to three or more classes of antimicrobial drugs based on susceptibility to erythromycin, clindamycin, chloramphenicol, ciprofloxacin, tetracycline, trimethoprim/ sulfamethoxazole [38].

Ethics Consideration: Ethics approval for this study was obtained from the University of Zambia Biomedical and Research Ethics Committee. Permission to use archived bacterial isolates was obtained from the management at the University Teaching Hospital. Study numbers were used to identify the bacterial isolates. Results for antimicrobial susceptibility testing were promptly reported to the attending physician for patient care.

Results

Forty-three percent (41/95) of the *S. aureus* isolates were identified as MRSA (Figure 1). The MRSA strains were predominantly multi-drug resistant, and were resistant to trimethoprim/sulfamethoxazole (100%), ciprofloxacin (95%), penicillin G (95%), erythromycin (78%), tetracycline (78%) and gentamicin (68%) (Figure 2). All the isolates were susceptible to vancomycin, teicoplanin and amikacin. Although only 2.5% of isolates were resistant to clindamycin by single-disc testing, the D-test identified erythromycin-induced clindamycin resistance in 68.3% of the isolates.

To assess whether antibiotic resistance phenotypes clustered together, antibiograms were

assigned using the designation TSGenEryCDCipCTe (TS for trimethoprim/ sulfamethoxazole; Gen for Gentamicin; Ery for Erythromycin; CD for Clindamycin; Cip for Ciprofloxacin; C for Chloramphenicol; and Te for Tetracycline), as shown in Table 1. Isolates could be grouped into 13 antibiogram patterns. Five predominant groups were

identified: TSGenEryCipT (23%), TSGenEryCipCT (18%), TSEryCip (15%), TSGenCipT (13%) and TSEryCipT (10%). Multi-drug resistance to a combination of four, five, six and seven antibiotics was observed in 17.5%, 27.5%, 35%, and 17.5% of the MRSA isolates, respectively (Table 2).

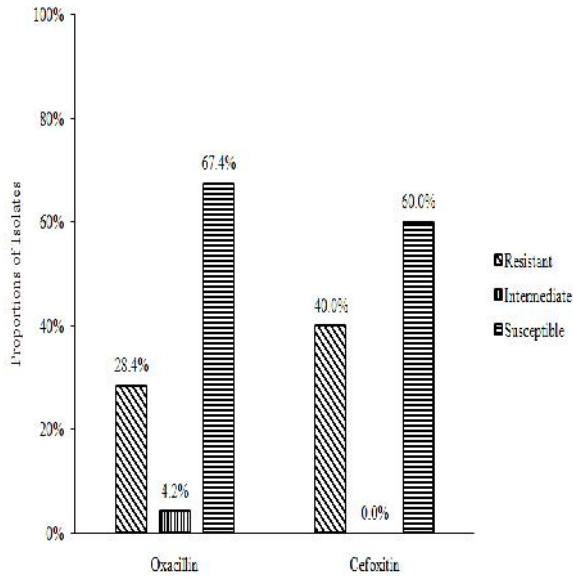


Figure 1: Detection of MRSA using 1µg Oxacillin and 30µg Cefoxitin discs

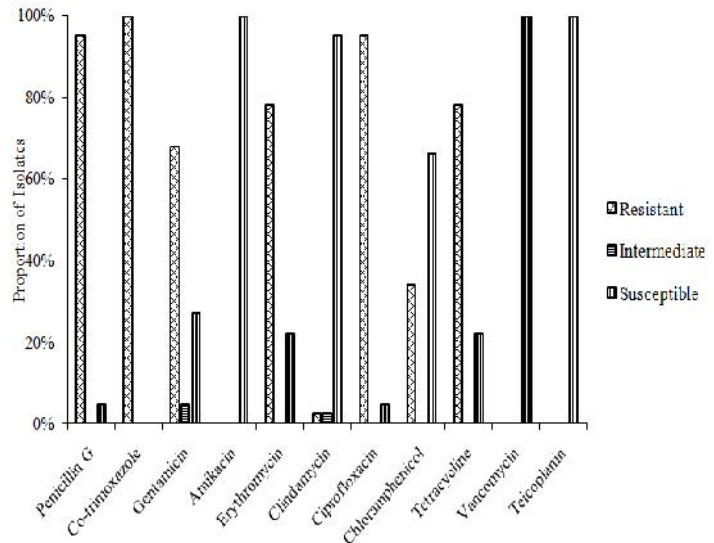


Figure 2: Susceptibility profiles of the 41 MRSA isolates from University Teaching Hospital

Resistance Pattern	No. of Isolates % (n)
TS + Gen + Ery + Cip + C + T	18 (7)
TS + Gen + Ery + CD + C + T	3 (1)
TS + Gen + Ery + Cip + T	23 (9)
TS + Gen + Cip + C + T	5 (2)
TS + Gen + Ery + Cip + C	3 (1)
TS + Ery + Cip + C + T	3 (1)
TS + Gen + Cip + T	13 (5)
TS + Ery + Cip + T	10 (4)
TS + Gen + Cip + C + T	3 (1)
TS + Ery + Cip + C + T	3 (1)
TS + Ery + Cip	15 (6)
TS + Gen + Cip	3 (1)
TS	3 (1)

Table 1: Antibiograms for MRSA Isolates; PG: Penicillin; TS: trimethoprim/sulfamethoxazole; Gen: Gentamicin; Ery: Erythromycin; CD: Clindamycin; Cip: Ciprofloxacin; C: Chloramphenicol; Te: Tetracycline.

Table 2: Frequency of Multi-drug Resistance among MRSA Isolates

No. of drug	Proportion. of Isolates % (n)
7	17.5 (7)
6	35 (14)
5	27.5 (11)
4	17.5 (7)

Discussion

Determination of antimicrobial susceptibility of bacterial pathogens using classical phenotypic tests in clinical microbiology is important in the therapeutic treatment of patients, which depends on assessing the susceptibilities of the bacteria [39]. In this study, the MRSA isolates were largely resistant to gentamicin, tetracycline, erythromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole.

Our results correspond more closely with reports from South Africa in which MRSA resistance rates to gentamicin (68% vs. 65.7%) and erythromycin (78% vs. 78.6%) were observed [30]. However, our study detected higher resistance rates to ciprofloxacin (95% vs 69.7%) and trimethoprim/sulfamethoxazole (100% vs. 55%). All isolates in our study were susceptible to amikacin, while the South African isolates were highly resistant (72%) to this drug.

Rates of resistance in this study were, in most cases, higher than those detected in another study conducted at the University Teaching Hospital [32]. However, the resistance to trimethoprim/sulfamethoxazole was comparable (100% vs 86%). Trimethoprim/sulfamethoxazole is one of the frequently used antibiotics for community-acquired MRSA treatment [24, 40, 41]. The high resistance to trimethoprim/sulfamethoxazole at the University Teaching Hospital could be due to widespread use of the drug in HIV-positive patients for the prophylaxis of pneumocystis, toxoplasma, and bacterial infections in sickle cell patients [42-44].

Clindamycin has been shown to be useful in the treatment of MRSA infections, especially for community-acquired infections [45-47]. However, inducible resistance is an important factor to consider when treating a patient with clindamycin [48-51]. In our study, 68.3% of the isolates showed inducible resistance to clindamycin. This indicates the importance of routine screening for inducible clindamycin resistance. No isolates resistant to vancomycin, teicoplanin and amikacin were detected in our study. However, since the disc diffusion method is not sensitive for detecting intermediate resistance, there is need for additional susceptibility testing to determine the true rates of glycopeptide resistance of MRSA at the University Teaching Hospital. Amikacin, though an old drug, would be available option for treatment of MRSA infections.

Of the 13 different antibiogram patterns determined in the study, 5 antibiogram patterns made up 79% of the isolates. Data on antibiotic patterns from different countries are usually very variable and difficult to compare. This is due to differences in the antimicrobial susceptibility testing methods used, and sometimes the number and type of antibiotics used. However, most importantly, it may also reflect differences in antimicrobial usage, and subsequent epidemiology of isolates at regional levels.

This study is not without limitations. As no clinical data to correspond with the MRSA isolates were available, we cannot infer the importance of our antimicrobial susceptibility data on the disease severity or treatment outcome. Furthermore, we could only analyse isolates obtained from the University Teaching Hospital as isolates from the other hospitals were not available. Therefore, further work is warranted to study *S. aureus* isolates from other parts of Zambia. This will give an accurate

picture of the burden of MRSA in the country. Additional studies are also needed to define the molecular epidemiology of this important pathogen.

Conclusion

MRSA isolates at the University Teaching Hospital in Lusaka show resistance to a number of antibiotics, and are multi-drug resistant. In particular, high resistance rates to trimethoprim/sulfamethoxazole and ciprofloxacin were detected. The low resistance to glycopeptides and clindamycin warrants the use of these drugs for the treatment of MRSA infections. However, since clindamycin inducible resistance was high, routine screening is recommended. This study provides baseline data for further antibiotic susceptibility studies of MRSA isolates at the University Teaching Hospital, and Zambia in general. Surveillance on resistance patterns and characterization of *S. aureus* in understanding new and emerging trends in Zambia is of paramount importance.

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Conflict of Interest: The contents of this paper reflect the views of the authors who are responsible for the facts and accuracy of the data presented herein and do not necessarily reflect the views or policies of any institution or agency. This paper does not constitute a standard, specification, nor is it intended for design, construction, bidding, contracting, or permit purposes. The authors have no conflicts of interest.

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