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Original Article

Antibiotics Resistance Pattern of *Staphylococcus aureus* Isolated From In-patients of an Orthopaedic Hospital in North-Western Nigeria

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Abstract

Background and aims: *Staphylococcus aureus*, an important pathogen in bone diseases, is a highly multi-drug resistant (MDR) bacterium. This study aimed to investigate the antibiotic resistance among *S. aureus* isolated from patients on admission in an orthopaedic hospital.

Methods: In this cross-sectional research, 140 samples comprising urine samples, wound swabs, and nasal swabs were collected from 49 patients on admission. Samples were cultured and screened for *S. aureus* following standard procedures. Using the agar-disk diffusion method, the isolates were subjected to antibiotics susceptibility tests.

Results: *S. aureus* were isolated from 26 (18.6%) samples, and wound swabs were found to have the highest number of the *S. aureus* isolates with 12 (46.2%). Among the 26 *S. aureus* isolated, 25 (96.2%) isolates were resistant to at least four or more of the tested antibiotics. There were 23 (88.5%) MDR isolates, while there were only 2 (7.6%) extensively drug resistant ones. The number of methicillin-resistant *S. aureus* were 17 (65.4% of the isolates), while the number of methicillin-susceptible *S. aureus* were 9 (34.6% of the isolates). A total of 22 (84.6%) isolates had multi-antibiotic resistance (MAR) index greater than 0.2. Inducible clindamycin resistance of 2 (7.6%) was observed.

Conclusion: This study showed that the *S. aureus* isolated from the patients were resistant to multiple antibiotics. Regular surveillance of antibiotic resistance is of utmost importance, since it facilitates the design or development of the treatment regimens that could check the spread of antimicrobial resistance.

Keywords: *Staphylococcus aureus*, Orthopaedic patients, Antibiotic resistance, Multi-drug resistance, North-Western Nigeria

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Introduction

Staphylococcus aureus is a facultative anaerobic bacterium that is frequently found in the respiratory tract and on the skin, mostly as a commensal organism.[1] This bacterium is responsible for about 70% of the osteomyelitis cases and 80% of joint infections in patients with rheumatoid arthritis and several other bone diseases.[2] A study on orthopaedic procedures generally demonstrated that nasal carriage of *S. aureus* increased the risk of *S. aureus* wound infection following orthopaedic surgery,[3] thereby causing long-term admission to health-care facility, which further increased the risk of orthopaedic surgical site infections. Bone infections caused by *S. aureus* are associated with rapid and localized destruction of the tissue.[4] Several studies have reported the internalization of *S. aureus* by both epithelial and endothelial cells.[5] Internalization of *S. aureus* by bone cells facilitates the progression of the disease via protecting the organism from extracellular host defences and/or antibiotic therapy.[4]

Antibiotics frequently used to treat *S. aureus* osteomyelitis include β-lactams antibiotics as well as clindamycin and fluoroquinolones. In addition, vancomycin, dicloxacillin, linezolid, daptomycin, and fosfomycin are used against resistant strains, such as methicillin resistant *S. aureus* (MRSA).[6] Furthermore, treatment of orthopaedic infections involves taking high doses of antibiotics in order to eradicate intracellular bacteria. However, long-term exposure of bacteria to antibiotics are the major cause of antibiotic resistance in bacteria; therefore, compounds that can synergistically improve the efficacy of antibiotics are needed.[4] Similarly, a major problem encountered in treatment of infections caused by *S. aureus* in human is their ability to acquire resistance to antibiotic rapidly. This resistance can be acquired by the help of mobile genetic elements such as plasmids, transposons, bacteriophages, pathogenicity islands, and staphylococcal cassette chromosomes that serve as the primary means by which genetic information are exchanged between bacteria via horizontal gene transfer.[7]

Staphylococcus aureus strains isolated from different clinical settings display significant genetic variations which

are responsible for the strain variation in physiology and stress response.[8] This includes the emergence of multiple drug-resistant *S. aureus* in hospitals and communities that form the basis for MRSA or more specifically hospital acquired-MRSA (HA-MRSA) and community acquired-MRSA (CA-MRSA) infections. The emergence of multi-drug resistant (MDR) strains of *S. aureus* has become a significant health threat since it has made the treatment of infections caused by these bacteria a great challenge. These MDR strains of *S. aureus* are considered a major risk in healthcare settings, and a similar increasing trend is observed in community-acquired infections.[9] Since the emergence of MRSA in 1960s, it has been disseminated globally and has become a leading cause of bacterial infections in both health-care and community settings.[10] In the United States, for instance, MRSA strains are the leading cause of death due to infections with a mortality rate of approximately 20%.[11] Methicillin-resistant *S. aureus* has been increasingly identified as a causative organism in health care associated infections, including orthopaedic infections.[12] Therefore, Mupirocin, which is an effective antibiotic against MRSA, may be used in the form of ointment for pre-operation and nasal decolonization of MRSA in patients in order to control the spread of MRSA among them during the outbreaks.[13]

Clindamycin is an excellent alternative antimicrobial agent that is used for patients with allergies to penicillin when treating localized and systemic infections caused by drug resistant *S. aureus*.[14] However, the inducible resistance to clindamycin in MRSA can severely compromise therapy and result in failure of clindamycin treatment of MRSA infections when non-suitable therapy like erythromycin is given.[15] Therefore, susceptibility testing for the detection of inducible resistance to clindamycin should be routinely performed. Inducible resistance phenotypes are those ones that are resistant to erythromycin or those that are with a clindamycin zone of inhibition ≥21 mm; these phenotypes are characterized by a D-shape zone of inhibition around clindamycin disk when placed at a distance of 12–20 mm away from an erythromycin disk on Mueller-Hinton agar plate. The constitutive resistance phenotypes, on the other hand, are those that are resistant to both erythromycin and clindamycin.[16]

This study aimed to investigate the antibiotics resistance profile of *S. aureus* isolated from patients on admission in an orthopaedic hospital in North-Western Nigeria.

Material and Methods

Sample Collection

A cross sectional design was employed in this study. Prior to the commencement of the study, ethical clearance was obtained from the institutional research board of the National Orthopaedic Hospital Dala (NOHD), in Dala Local Government of Kano State, Nigeria to enable collection of clinical specimen from the hospitalized orthopaedic patients. In addition, only patients who gave us informed consent were included in the study.

Overall, a total of 140 clinical specimens were obtained from 49 patients between September and December, 2017. These samples were comprised of 49 wound swabs, 49 nasal swabs, and 42 urine samples. The specimens were transported to the Microbiology Laboratory, Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria using a sterile Ziploc plastic bag for microbiological evaluation.

Bacterial Isolation and Identification

The clinical samples were inoculated into freshly prepared nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated at 37°C for 24 hours. A loop-full of growth from the various nutrient broth media were streaked onto Mannitol Salt Agar (Oxoid Ltd., Basingstoke, Hampshire, England) plates and incubated for 24 hours at 37°C, as described by UK Standards.[17] Discrete, single, and golden-yellow colonies were presumptively identified as Staphylococci.

Isolates were identified preliminarily using methods described by Acharya and Aditi et al to determine their gram reaction and catalase test, respectively.[18,19] Coagulase test was done on the isolates to differentiate *S. aureus* (coagulase positive) from coagulase negative *Staphylococcus* (CONS) using Dry Spot Staphytech Plus Test Kit.

To identify and differentiate the specie of the *Staphylococcus* isolated, those isolates that were Gram-positive cocci, catalase positive, and coagulase positive were subjected to commercially available Microgen™ Staph-ID System test that uses 12 standardized biochemical substrate in micro-wells to identify medically important members of the genus *Staphylococcus*.

Determination of Antibiotics Resistance of the Staphylococcus aureus Isolates

Using the agar-disc diffusion method described in EUCAST,[20] antibiotic resistance was performed on the *S. aureus* isolates (Figure 1) Twelve antibiotics from the classes of antibiotics used for treatment of *S. aureus* were: Cefoxitin (30 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), norfloxacin (10 µg), ciprofloxacin (10 µg), linezolid (30 µg), quinupristin-dalfopristin (15 µg), mupirocin (5 µg and 200 µg), tetracycline (30 µg), amoxicillin/clavulanic acid (30 µg) and trimethoprim-

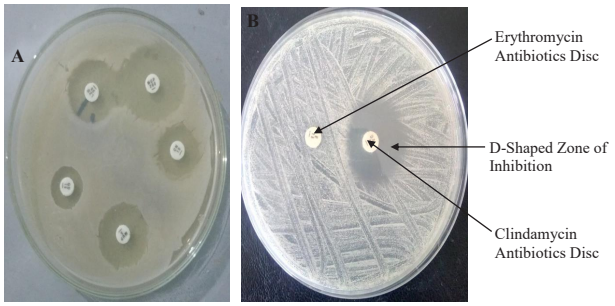

Figure 1. (A) Antibiotic Susceptibility Test (B) Inducible Clindamycin D-Test.

sulfamethoxazole (1.25 µg + 23.75 µg) (Oxoid Ltd., Basingstoke, Hampshire, England).

Determination of Inducible Clindamycin Resistance in Staphylococcus aureus Isolates

Those isolates that showed resistance to erythromycin (no zone of inhibition) but were susceptible (showed zone of inhibition) to clindamycin were subjected to inducible clindamycin resistance test. The D-zone test method was used according to EUCAST[20] guidelines, where erythromycin (15 µg) disc was placed at a distance of 12–20 mm edge-to-edge from clindamycin (2 µg) disc on a Mueller Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England) plate inoculated with 0.5 McFarland standard equivalent bacterial suspensions. After incubation at 37°C for 18 hours, flattening of clindamycin zone of inhibition adjacent to the erythromycin disc (referred to as a D-zone) indicated erm-mediated inducible clindamycin resistance (positive D-test) (Figure 1).[21]

Determination of Resistance Class of the *Staphylococcus aureus* Isolates

The definitions for multiple drug resistance proposed by the European Center for Disease prevention and Control/ European Medicines Agency (ECDC/EMEA) joint technical report was used. The resistance shown by the *S. aureus* isolates in this study were classified into MDR and extensively drug resistant (XDR).[22,23]

Determination of Multi-Antibiotics Resistance Index of the Staphylococcus aureus Isolates

This was carried out as described with slight modification using the formula "MAR index=resistant antibiotics divided by the total tested antibiotics.[24,25] It further states that MAR index values >0.2 indicate existence of isolate from high-risk contaminated source with frequent use of antibiotics, while values ≤0.2 show bacteria from source with less antibiotics usage.

Data Analysis

Data were presented in percentage and the results from the antibiotic resistance profile of the *S. aureus* were presented in tables and bar chart.

Results

Isolation of Staphylococcus aureus

Out of the various samples collected from the patients on admission in the hospital, 26 (18.6%) of *S. aureus* were isolated from various sample types. Majority of *S. aureus* isolates (50%) were from the male population aged between 18-40 years, and the wound swabs had the highest prevalence of *S. aureus* among the sample types (46.2%), as shown in Tables 1 and 2, respectively.

Antibiotics Resistance Profile of the Staphylococcus aureus Isolates

As shown in Figure 2, the *S. aureus* isolates were generally resistant to amoxicillin-clavulanic acid, tetracycline, ciprofloxacin and cefoxitin, norfloxacin, clindamycin, gentamicin, erythromycin, trimethoprim-sulfamethoxazole, quinupristin-dalfopristin, mupirocin and linezolid. Resistance to cefoxitin was an indicator for MRSA.

Antibiotics Resistance Pattern

The *S. aureus* isolates have different patterns of antibiotic resistance (as shown in Table 3) and are classified based on these patterns. All isolates have different antibiotic patterns.

Classes of Resistance in the *Staphylococcus aureus* Isolates

The antibiotic resistance classifications are shown in Figure 3. All the *S. aureus* isolates were resistant to at least one of the test antibiotics, although 1 (3.9%) was susceptible to all the antibiotics that were tested

Distribution of Methicillin Resistant Staphylococcus aureus in the Patients

In this study, the resistance to Cefoxitin was used to classify the isolates as MRSA and methicillin susceptible *S. aureus* (MSSA). Out of the 26 *S. aureus* isolates, 17 (65.4%) were MRSA, while 9 (34.6%) were MSSA. Figure 4 shows the distribution of MRSA and MSSA isolates according to their sample sources.

Multi-Antibiotics Resistance Index of the Staphylococcus aureus Isolates

The multi-antibiotic resistance (MAR) index was determined as the ratio of the number of antibiotics to which the *S. aureus* isolates were resistant, to the total number of antibiotics to which the organisms were exposed. A total of 84.6% of the isolates had MAR index >0.2, as shown in Table 4.

Table 1. Percentage Distribution of *Staphylococcus aureus* Isolates by Age-Group and Gender of Patients

Age Range (y)	Percentage of Patients Recruited		All Patients
	Male	Female	
1–17	2 (7.6%)	1 (3.9%)	3 (11.5%)
18–40	13 (50%)	3 (11.5%)	16 (61.5%)
41–above	6 (23.1%)	1 (3.9%)	7 (27%)
Total	21 (80.7%)	5 (19.3%)	26 (100%)

Table 2. Distribution of the *Staphylococcus aureus* Isolates by Specimen

Isolate Source	*Staphylococcus aureus* n (%)
Wound swab	12 (46.1%)
Nasal swab	8 (30.8%)
Urine sample	6 (23.1%)
Total	26 (100%)

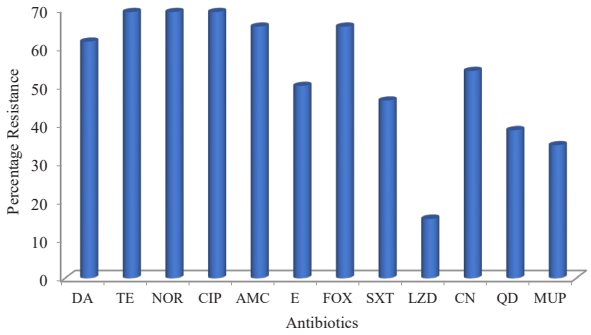

Figure 2. Antibiotics Resistance of *Staphylococcus aureus* Isolates to Tested Antibiotics. Abbreviations: DA, Clindamycin; TE, Tetracycline; NOR, Norfloxacin; CIP, Ciprofloxacin; AMC, Amoxicillin-clavulanic acid; E, Erythromycin; FOX, Cefoxitin; SXT, Trimethoprim-sulfamethoxazole; LZD, Linezolid; CN, Gentamicin; QD, Quinupristin-dalfopristin and MUP, Mupirocin.

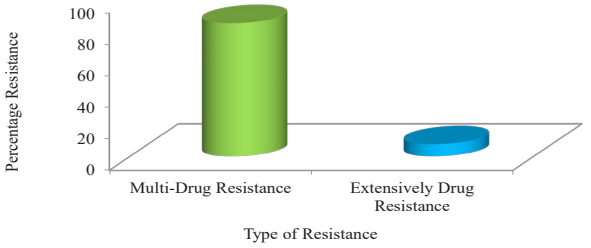

Figure 3. Percentage Distribution of Resistance Types in the *Staphylococcus aureus* Isolates

Inducible Clindamycin Resistance in the Staphylococcus aureus Isolates

According to the results of the antibiotic susceptibility tests, 10 isolates (38.5%) out of 26 were susceptible to clindamycin, while a total of 16 isolates (61.5%) were resistant. Inducible clindamycin test (D-Test) was carried out on 5 *S. aureus* isolates that showed resistance to erythromycin (i.e. no zone of inhibition), but they were found susceptible to clindamycin (i.e., showed zone of inhibition). The result further demonstrated that 2 isolates out of the 16 resistant were inducible clindamycin resistant isolates, and 14 were constitutively resistant to clindamycin. This is shown in Figure 5.

Discussion

In this study, 140 collected clinical samples were examined and *S. aureus* were recovered from 26 of them (18.6%). Most of the *S. aureus* isolates were obtained from wound swab since the wound, compared to the nostril and urine, provides more conducive environment for proliferation of *S. aureus* as a haemolytic organism. However, the study of Dilnessa and Bitew[26] generated different results in terms of their sources. According to their study results, nasal swab had the highest prevalence of 33.3% compared to 30.8% nasal swab prevalence found in our study. Contrary to this study, Ibrahim et al[27] reported *S. aureus* isolation rate of 47.3% in the same geographical area (Kano, Nigeria), with the isolates from wound swabs also having the highest prevalence (32.7%).

The *S. aureus* isolated in this study were found highly resistant to the tested antimicrobial agents. The resistance rate of the *S. aureus* isolates was above 60% for tetracycline, norfloxacin, ciprofloxacin, amoxicillin-clavulanic acid, and cefoxitin. The *S. aureus* isolates showed least resistance to linezolid (15.4%). This rate was lower than the one (24%) reported in a study from India.[28] Linezolid, the most effective antimicrobial agent used in this study, was listed among the drugs approved for treatment of MRSA infections,[7] and was recommended as the drug of last resort in severe cases of MRSA. Classification of the resistance profile of the isolates as MDR (88.5%) and XDR (7.6%) in this study revealed the risk posed by infections caused by these isolates, which may have resulted in treatment failure, prolonged hospitalisation, and increased burden associated with health-care costs. The prevalence of MDR in this study was twice as much the prevalence reported by Basak et al in India (37.1%), while the XDR prevalence was half that reported in the Indian study (13.8%); no PDR was observed among the tested bacterial strains.[29] Even though resistance profiles were expected to vary among studies from different communities, a study conducted in Limpopo province, South Africa documented a high antibiotic resistance among *S. aureus* isolates.[30]

Using cefoxitin breakpoint as an indicator for methicillin resistance in *S. aureus* as recommended by EUCAST,[20] this study showed that 65.4% of the *S. aureus* isolates were MRSA, while 34.6% of them were MSSA. This high MRSA may have been due to the prolonged hospitalization, open wounds, long-term indwelling catheter, and living in areas

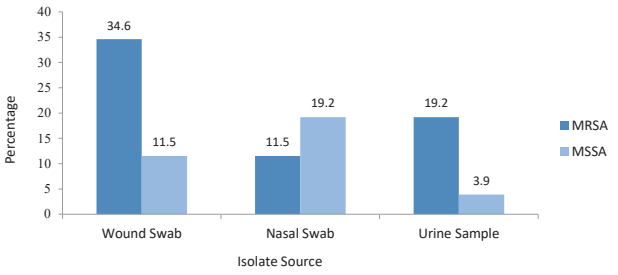

Figure 4. Distribution of Methicillin-Resistant *Staphylococcus aureus* and Methicillin Susceptible *Staphylococcus aureus* by Sample Source.

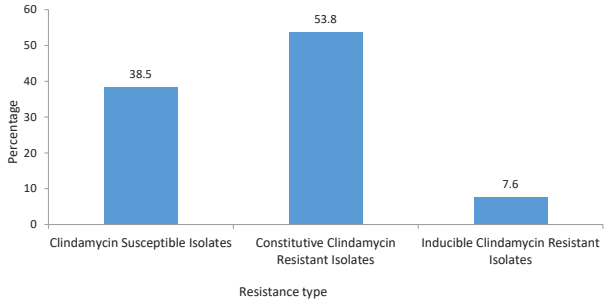

Figure 5. Percentage Inducible Clindamycin Resistance in the *Staphylococcus aureus* Isolates.

Table 3. Antibiotics Resistance Phenotype in *Staphylococcus aureus* Isolates

Resistance Phenotype	No. of Isolates (n=26)	Percent
TE	1	3.9
DA, TE	1	3.9
DA, MUP	1	3.9
DA, TE, AMC, E, QD	1	3.9
CIP, AMC, FOX, MUP	1	3.9
DA, TE, NOR, CN, QD	2	7.6
TE, NOR, CIP, FOX, SXT	1	3.9
NOR, CIP, AMC, E, FOX, CN	1	3.9
NOR, CIP, FOX, SXT, CN, QD	1	3.9
DA, TE, CIP, AMC, FOX, MUP	1	3.9
DA, AMC, FOX, SXT, LZD, MUP	1	3.9
DA, TE, NOR, CIP, AMC, E, FOX	1	3.9
TE, NOR, CIP, AMC, E, FOX, SXT	1	3.9
TE, NOR, CIP, AMC, FOX, SXT, CN	1	3.9
TE, NOR, CIP, AMC, E, FOX, CN, QD	1	3.9
TE, NOR, CIP, AMC, E, FOX, SXT, CN	1	3.9
DA, NOR, CIP, AMC, E, FOX, SXT, CN	1	3.9
DA, TE, NOR, CIP, AMC, FOX, SXT, CN	1	3.9
DA, TE, NOR, CIP, AMC, E, FOX, SXT, CN	1	3.9
DA, TE, NOR, CIP, AMC, E, FOX, QD, MUP	1	3.9
DA, NOR, CIP, AMC, E, FOX, CN, QD, MUP	1	3.9
DA, TE, NOR, CIP, E, SXT, LZD, CN, QD, MUP	1	3.9
DA, TE, NOR, CIP, AMC, E, SXT, LZD, CN, QD, MUP	1	3.9
DA, TE, NOR, CIP, AMC, E, FOX, SXT, LZD, CN, QD, MUP	1	3.9

Abbreviations: DA, Clindamycin; TE, Tetracycline; NOR, Norfloxacin; CIP, Ciprofloxacin; AMC, Amoxicillin-clavulanic acid; E, Erythromycin; FOX, Cefoxitin; SXT, Trimethoprim-sulfamethoxazole; LZD, Linezolid; CN, Gentamicin; QD, Quinupristin-dalfopristin and MUP, Mupirocin.

Table 4. Multi-Antibiotic Resistance index of *Staphylococcus aureus* Isolates

MAR index	No. of isolates	Percent
0.08	1	3.8
0.17	3	11.5
0.25	1	3.8
0.33	4	15.4
0.42	7	26.9
0.50	4	15.4
0.58	4	15.4
0.66	1	3.8
0.75	1	3.8

or staying in hospitals with high prevalence of CA-MRSA and HA-MRSA.[31] In a study by Nwankwo et al in Kano, Nigeria, the MRSA prevalence among in-patients was found to be 62%,[32] which was lower than that detected in this study; while Udobi et al[33] reported a higher MRSA prevalence of 75% for Staphylococcal isolates from the orthopaedic wards of Ahmadu Bello University Teaching Hospital Zaria, Nigeria.

MAR index was a very helpful tool for assessing the potential health risk of the *S. aureus* isolates. In this study, 84.6% of the isolates had MAR index > 0.2. Bacteria having MAR index >0.2 indicate origins from areas of selective pressure due to frequent and possible antibiotic abuse, while values ≤0.2 shows that bacteria are from sources with low antibiotic usage.[24,25] This suggests that the 84.6% of *S. aureus* isolates with MAR index >0.2 spread from a niche of high antibiotic use. Hence, the selection of antibiotics becomes more difficult. This calls for vigilant surveillance and remedial measures.

Inducible clindamycin resistance in this study was discovered to make up 7.6% of the *S. aureus* isolates, and may have possessed the *erm* gene encoded for macrolide, lincosamide, and streptogramins resistance. All isolates showing inducible clindamycin resistance were resistant to cefoxitin. clindamycin is a reserve drug and, depending on the antimicrobial susceptibility results, its application is usually advocated when treating severe in-patient MRSA infection. In addition, proper use of clindamycin in severe MRSA can reduce the use of vancomycin [14] and it is known to possess exceptionally high bone penetration.[34] Therefore, the inducible clindamycin results from this study suggested that under strict supervision, clindamycin may have been used for patients treating other infections with erythromycin.

This study also found 34.6% mupirocin resistance among the isolates. As for the mupirocin resistant isolates, 77.8%

of them were resistant to cefoxitin. As such, mupirocin ointment may not have been the best choice for pre-operation and nasal decolonization of MRSA for patients. Mupirocin resistance in *S. aureus* isolates is usually an indication of *mupA* gene carriage in their genome.[35]

Conclusion

According to our study findings, the *S. aureus* isolates were highly MDR since they showed high resistance to tetracycline, norfloxacin, ciprofloxacin, amoxicillin-clavulanic acid, and Cefoxitin; however, they were most susceptible to linezolid. It was recommended that the utmost importance be attached to the regular surveillance of antibiotic resistance, since it aided the design or development of the treatment regimens capable of checking the spread of antimicrobial resistance.

Ethical Approval

Ethical approval was sought and obtained from the Ethical Committee of the National Orthopaedic Hospital, Dala (NOHD).

Conflict of Interest Disclosures

The authors declare that there is no conflict of interests.

Acknowledgement

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References

1. Asadi S, Jamali M. Assessment the frequency of *Staphylococcus aureus* golden methicillin-resistant (MRSA) and vancomycin-resistant VRSA in determining the MIC using E-test. Immunol Disord Immunother. 2017;2(1):104. doi: 10.35248/2593-8509.17.2.112.
2. Ashong CN, Raheem SA, Hunter AS, Mindru C, Barshes NR. Methicillin-resistant *Staphylococcus aureus* in foot osteomyelitis. Surg Infect (Larchmt). 2017;18(2):143-8. doi: 10.1089/sur.2016.165.
3. Crowe B, Payne A, Evangelista PJ, Stachel A, Phillips MS, Slover JD, et al. Risk factors for infection following total knee arthroplasty: a series of 3836 cases from one institution. J Arthroplasty. 2015;30(12):2275-8. doi: 10.1016/j.arth.2015.06.058.
4. Dusane DH, Kyrouac D, Petersen I, Bushrow L, Calhoun JH, Granger JF, et al. Targeting intracellular *Staphylococcus aureus* to lower recurrence of orthopaedic infection. J Orthop Res. 2018;36(4):1086-92. doi: 10.1002/jor.23723.
5. Sheehy SH, Atkins BA, Bejon P, Byren I, Wyllie D, Athanasou NA, et al. The microbiology of chronic osteomyelitis: prevalence of resistance to common empirical anti-microbial regimens. J Infect. 2010;60(5):338-43. doi: 10.1016/j.jinf.2010.03.006.
6. Ambrosch A, Haefner S, Jude E, Lobmann R. Diabetic foot infections: microbiological aspects, current and future antibiotic therapy focusing on methicillin-resistant *Staphylococcus aureus*. Int Wound J. 2011;8(6):567-77. doi: 10.1111/j.1742-481X.2011.00849.x.
7. McGuinness WA, Malachowa N, DeLeo FR. Vancomycin resistance in *Staphylococcus aureus*. Yale J Biol Med. 2017;90(2):269-81.
8. Sabirova JS, Xavier BB, Hernalsteens JP, De Greve H, Ieven M, Goossens H, et al. Complete genome sequences of two prolific biofilm-forming *Staphylococcus aureus* isolates belonging to USA300 and EMRSA-15 clonal lineages. Genome Announc. 2014;2(3). doi: 10.1128/genomeA.00610-14.
9. Kadariya J, Thapaliya D, Bhatta S, Mahatara RL, Bempah S, Dhakal N, et al. Multidrug-resistant *Staphylococcus aureus* colonization in healthy adults is more common in Bhutanese refugees in Nepal than those resettled in Ohio. Biomed Res Int. 2019;2019:5739247. doi: 10.1155/2019/5739247.
10. Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, et al. Methicillin-resistant *Staphylococcus aureus*. Nat Rev Dis Primers. 2018;4:18033. doi: 10.1038/nrdp.2018.33.
11. Dean MA, Olsen RJ, Long SW, Rosato AE, Musser JM. Identification of point mutations in clinical *Staphylococcus aureus* strains that produce small-colony variants auxotrophic for menadione. Infect Immun. 2014;82(4):1600-5. doi: 10.1128/iai.01487-13.
12. Kawamura H, Matsumoto K, Shigemi A, Orita M, Nakagawa A, Nozima S, et al. A bundle that includes active surveillance, contact precaution for carriers, and cefazolin-based antimicrobial prophylaxis prevents methicillin-resistant *Staphylococcus aureus* infections in clean orthopedic surgery. Am J Infect Control. 2016;44(2):210-4. doi: 10.1016/j.ajic.2015.09.014.
13. Anderson DJ, Podgorny K, Berríos-Torres SI, Bratzler DW, Dellinger EP, Greene L, et al. Strategies to prevent surgical site infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol. 2014;35 Suppl 2:S66-88. doi: 10.1017/s0899823x00193869.
14. Paul R, Pal L, Saha R, Shaw A, Kumar N. Prevalence of inducible clindamycin resistance and methicillin resistance among *Staphylococcus* species from various clinical samples in a tertiary care hospital of eastern India. Ann Int Med Dent Res. 2019;5(5):1.
15. Ahmed MO, Abuzweda AR, Alghazali MH, Elramalli AK, Amri SG, Aghila E, et al. Misidentification of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals in Tripoli, Libya. Libyan J Med. 2010;5. doi: 10.3402/ljm.v5i0.5230.
16. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100. CLSI; 2018.
17. UK Standards for Microbiology Investigations. Inoculation of culture media for bacteriology. Available from: https://www.gov.uk/government/publications/smi-q-5-inoculation-of-culture-media-for-bacteriology.
18. Tankeshwar A. Gram Staining: Principle, Procedure and Results. https://microbeonline.com/gram-staining-principle-procedure-results/.
19. Aditi FY, Rahman SS, Hossain MM. A study on the microbiological status of mineral drinking water. Open Microbiol J. 2017;11:31-44. doi: 10.2174/1874285801711010031.
20. European Committee on Antimicrobial Susceptibility Testing. Breaking points tables for interpretation of MICs and zone diameters. Version 6.0. 2018. http://www.eucast.org.
21. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol. 2007;56(Pt 3):342-5. doi: 10.1099/jmm.0.46761-0.
22. European Center for Disease Prevention and Control (ECDC), European Medicines Agency (EMA). ECDC/EMEA Joint Technical Report: The Bacterial Challenge: Time to React. Stockholm: ECDC, EMA; 2009.
23. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.

25. Thenmozhi S, Rajeswari P, Kumar BS, Saipriyanga V, Kalpana M. Multi-drug resistant patterns of biofilm forming *Aeromonas hydrophila* from urine samples. Int J Pharm Sci Res. 2014;5(7):2908-18. doi: 10.13040/ijpsr.0975-8232.5(7).2908-18.
26. Dilnessa T, Bitew A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. BMC Infect Dis. 2016;16:398. doi: 10.1186/s12879-016-1742-5.
27. Ibrahim S, Adam AS, Aliero AA, Umar S. Prevalence and antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from wound and otitis media among patients attending Aminu Kano Teaching Hospital, Kano, Nigeria. Microbiol Res J Int. 2018;25(2):1-9. doi: 10.9734/mrji/2018/44684.
28. Thool VU, Bhoosreddy GL, Wadher BJ. Detection of resistance to linezolid in *Staphylococcus aureus* infecting orthopedic patients. Indian J Pathol Microbiol. 2012;55(3):361-4. doi: 10.4103/0377-4929.101745.
29. Basak S, Singh P, Rajurkar M. Multidrug resistant and extensively drug resistant bacteria: a study. J Pathog. 2016;2016:4065603. doi: 10.1155/2016/4065603.
30. Samie A, Shivambu N. Biofilm production and antibiotic susceptibility profiles of *Staphylococcus aureus* isolated from HIV and AIDS patients in the Limpopo province, South Africa. Afr J Biotechnol. 2011;10(65):14625-36. doi: 10.5897/ajb11.1287.
31. Siddiqui AH, Koirala J. Methicillin resistant *Staphylococcus aureus* (MRSA). In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2018.
32. Nwankwo BO, Abdulhadi S, Magagi A, Ihesiulor G. Methicillin resistant *S. aureus* (MRSA) and their antibiotic sensitivity pattern in Kano, Nigeria. Afr J Clin Exp Microbiol. 2010;11(1):29-33. doi: 10.4314/ajcem.v11i1.44088.
33. Udobi CE, Obajuluwa AF, Onaolapo JA. Prevalence and antibiotic resistance pattern of methicillin-resistant *Staphylococcus aureus* from an orthopaedic hospital in Nigeria. Biomed Res Int. 2013;2013:860467. doi: 10.1155/2013/860467.
34. Landersdorfer CB, Bulitta JB, Sörgel F. Pharmacokinetics and pharmacodynamics of antibiotics in bone. In: Zimmerli W, ed. Bone and Joint Infections: From Microbiology to Diagnostics and Treatment. Chichester: John Wiley & Sons Ltd; 2015. p.21-37.
35. Park SH, Kim JK, Park K. In vitro antimicrobial activities of fusidic acid and retapamulin against mupirocin- and methicillin-resistant *Staphylococcus aureus*. Ann Dermatol. 2015;27(5):551-6. doi: 10.5021/ad.2015.27.5.551.