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

**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. 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. A study on orthopaedic procedures generally demonstrated that nasal carriage of *S. aureus* increased the risk of *S. aureus* wound infection following orthopaedic surgery, 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. Several studies have reported the internalization of *S. aureus* by both epithelial and endothelial cells. 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.

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). 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. 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.
are responsible for the strain variation in physiology and stress response. 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. 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. In the United States, for instance, MRSA strains are the leading cause of death due to infections with a mortality rate of approximately 20%. Methicillin-resistant S. aureus has been increasingly identified as a causative organism in healthcare-associated infections, including orthopaedic infections. 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.

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. However, the inducible resistance to clindamycin in MRSA can severely compromise therapy and result in failure of clindamycin treatment of MRSA infections when non-suited therapy like erythromycin is given. 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.

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. 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. 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, 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), norfloxacain (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-

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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 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=number of antibiotics to which the organisms were resistant divided by the total number of antibiotics”24,25. It further states that MAR index values >0.2 indicate existence of isolates with 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>
<thead>
<tr>
<th>Age Range (y)</th>
<th>Percentage of Patients Recruited</th>
<th>All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1–17</td>
<td>2 (7.6%)</td>
<td>1 (3.9%)</td>
</tr>
<tr>
<td>18–40</td>
<td>11 (50%)</td>
<td>3 (11.5%)</td>
</tr>
<tr>
<td>41–above</td>
<td>6 (23.1%)</td>
<td>1 (3.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>21 (80.7%)</td>
<td>5 (19.3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolate Source</th>
<th>Staphylococcus aureus n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound swab</td>
<td>12 (46.1%)</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>8 (30.8%)</td>
</tr>
<tr>
<td>Urine sample</td>
<td>6 (23.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (100%)</td>
</tr>
</tbody>
</table>

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

Table 2. Distribution of the Staphylococcus aureus Isolates by Specimen
Distribution of Methicillin-Resistant isolates 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,26 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 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 Bitew27 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 al27 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

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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.
or staying in hospitals with high prevalence of CA-MRSA and HA-MRSA. In a study by Nwankwo et al in Kano, Nigeria, the MRSA prevalence among in-patients was found to be 62%, which was lower than that detected in this study; while Udobi et al 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. 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 and it is known to possess exceptionally high bone penetration. 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%

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

<table>
<thead>
<tr>
<th>Resistance Phenotype</th>
<th>No. of Isolates (n=26)</th>
<th>Percent</th>
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<tbody>
<tr>
<td>TE</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>DA, TE</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>DA, MUP</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>DA, TE, AMC, E, QD</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>CIP, AMC, FOX, MUP</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>DA, TE, NOR, CN, QD</td>
<td>2</td>
<td>7.6</td>
</tr>
<tr>
<td>TE, NOR, CIP, FOX, SXT</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>NOR, CIP, AMC, E, FOX, CN</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>NOR, CIP, FOX, SXT, CN, QD</td>
<td>1</td>
<td>3.9</td>
</tr>
<tr>
<td>DA, TE, CIP, AMC, FOX, MUP</td>
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<td>3.9</td>
</tr>
<tr>
<td>DA, AMC, FOX, SXT, LZD, MUP</td>
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<td>3.9</td>
</tr>
<tr>
<td>DA, TE, NOR, CIP, AMC, E, FOX</td>
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<td>3.9</td>
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<tr>
<td>DA, NOR, CIP, AMC, E, FOX, SXT, CN</td>
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<td>3.9</td>
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<tr>
<td>DA, NOR, CIP, AMC, E, FOX, SXT, LZD, CN, QD, MUP</td>
<td>1</td>
<td>3.9</td>
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</table>

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

<table>
<thead>
<tr>
<th>MAR index</th>
<th>No. of isolates</th>
<th>Percent</th>
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<tbody>
<tr>
<td>0.08</td>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>0.17</td>
<td>3</td>
<td>11.5</td>
</tr>
<tr>
<td>0.25</td>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>0.33</td>
<td>4</td>
<td>15.4</td>
</tr>
<tr>
<td>0.42</td>
<td>7</td>
<td>26.9</td>
</tr>
<tr>
<td>0.50</td>
<td>4</td>
<td>15.4</td>
</tr>
<tr>
<td>0.58</td>
<td>4</td>
<td>15.4</td>
</tr>
<tr>
<td>0.66</td>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>0.75</td>
<td>1</td>
<td>3.8</td>
</tr>
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</table>
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


