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# Occurrence of Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococcus Aureus* in Some Selected Hospitals of Kano Metropolis, Nigeria

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## Abstract

*Methicillin-resistant Staphylococcus aureus (MRSA) is a major public health concern worldwide, responsible for infections in both healthcare settings and the general population. The emergence of multidrug-resistant MRSA strains, including those resistant to macrolides and lincosamides, poses a serious threat to effective treatment due to this bacterial infection. This study aimed to determine the occurrence and resistance pattern of* Staphylococcus aureus *resistant to methicillin and inducible clindamycin resistance in some clinical isolates from selected hospitals in Kano metropolis, Kano State, Nigeria. Clinical isolates of MRSA were collected from patients diagnosed with various clinical infections in selected hospitals in Kano Metropolis. Antimicrobial susceptibility testing was performed*

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*using standard methods, and the presence of inducible clindamycin resistance genes (erm genes) was detected using D-test detection technique. One hundred and fifty (150) clinical isolates of* S. aureus *were collected for the purpose of this study. The prevalence rate of 39.3% MRSA and 12.0% inducible clindamycin resistance strain among these isolates was obtained. Cotrimoxazole was found to be the most resistant antibiotic against the isolated staphylococcus species in the study. The findings of this study highlight the emergence of MRSA and inducible clindamycin-resistant* S. aureus *in the study area. Continuous surveillance and monitoring of antimicrobial resistance patterns are essential for effective management of MRSA infections in this region.*

**Keywords**: *Erm* genes, Kano Metropolis, Macrolide-lincosamide resistance, Methicillin-resistant Staphylococcus aureus, Nigeria.

## INTRODUCTION

Since the first discovery of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in the early 1960s, it has become one of the major public health challenges due to its widespread and several clones (Romero-Gomez *et al.,* 2013). Methicillin-resistant *Staphylococcus aureus* has become one leading cause of hospital-acquired infections worldwide accounting for more than 60% of *S. aureus* isolates in hospitals in the United States (Wangai *et al.*, 2019). MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many other chemotherapeutic agents such as the quinolones, aminoglycosides, and a low level resistant to vancomycin (Kaur and Chate, 2015). The acquisition of such resistance does not necessarily cause the organism to be more intrinsically virulent than other strains of *S. aureus* that have no antibiotic resistance, but it does make MRSA infections more difficult to treat with standard types of antibiotics and thereby more dangerous (Idris *et al.*, 2019).

Emerging resistance to methicillin in Staphylococci has left us with very few therapeutic alternatives to treat the infections caused by this organism. Clindamycin in macrolide-lincosamide streptogramin B ($MLS_B$) family of antibiotics serves as one such alternative for treating both methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) infections, due to its excellent pharmacokinetic properties (Adhikari *et al.*, 2017). However, the extensive use to this class of antibiotic resulted in the emergence of numerous staphylococcal strains resistant to it. Resistance to $MLS_B$ antibiotics arises through various mechanisms (Adhikari *et al.*, 2017).

Although antibiotics like vancomycin, linezolid, and quinupristin-dalfopristin are often the preferred treatment options, *Staphylococcus aureus* strains with reduced susceptibility or resistance to these agents have emerged (Shariati *et al.*, 2020). Consequently, macrolide-lincosamide-streptogramin B (MLSB) antibiotics have been considered as alternative therapies for such infections. However, previous studies have documented both constitutive and inducible clindamycin resistance in *S. aureus* strains, primarily due to the widespread use of MLSB antibiotics. Therefore, it is essential to carefully monitor and detect both forms of clindamycin resistance to avoid therapeutic failure in treating patients with *S. aureus* infections.

## MATERIAL AND METHODS

### Study Area

The study was carried out in two selected hospitals in Kano metropolis that included Murtala Muhammad Specialist Hospital (MMSH) located at Kano Municipal Local Government Area,

that has 500 bed capacity, and Muhammad Abdullahi Wase Teaching Hospital, Kano (MAWTH) located at Nasarawa Local Government Area with 200 bed capacity (Alkali *et al.*, 2019), all in Kano metropolis, at latitudes $10^{o}$ 33N to $11^{o}$ 15N and longitudes $34^{o}$CE to $8^{o}$ 20CE (NBS, 2018).

**Study Design**
The study was a cross sectional hospital-based study and consist of Staphylococcal isolates obtained from clinical isolates.

**Ethical Approval**
The ethical permission was obtained from the Kano State Ministry of Health, before the commencement of the study (REF No: NHREC/17/03/2018).

**Sample Collection**
A total of 150 *Staphylococcal* isolates were collected from various clinical samples, including blood cultures, wound and burn swabs, urine, cerebrospinal fluid, surgical site infections, ear swabs, eye swabs, throat swabs, urethral swabs, and abscesses. Of these, 89 *Staphylococcal* isolates were collected from MMSH, and 61 were from WAHTH, all obtained from the Microbiology Laboratories of both sites.

**Bacteriological Analysis**

**Sample Processing**
All collected samples were subcultured on Blood Agar and Mannitol Salt Agar to confirm the isolates and obtain pure colonies for further analysis. All media were prepared according to the manufacturer's instructions.

**Gram Staining Reaction**
Gram staining of the isolated colonies was performed to determine the Gram's reaction. A thin smear of the isolate was prepared on a clean glass slide with sterile normal saline and allowed to air dry. The smear was heat-fixed, stained with crystal violet for 30 seconds, followed by iodine as a mordant, then decolorized with 90% alcohol. Safranin was applied as the secondary stain for 30 seconds. After washing with water at each stage, the slide was air-dried and observed under an oil immersion lens (×100) for Gram reaction (Cheesbrough, 2010).

**Biochemical Tests**
Suspected *Staphylococcal* isolates from various samples were further identified using standard bacteriological procedures, including the catalase test, coagulase test, hemolysis activity, and mannitol fermentation (Cheesbrough, 2010).

**Catalase Test**
The catalase test was conducted by adding 2ml of hydrogen peroxide ($H_2O_2$) to a test tube. Fresh colonies of the test organism, less than 24 hours old, were introduced into the solution using a sterile wire loop. Immediate observation for bubble ($O_2$) formation indicated a positive catalase reaction (Cheesbrough, 2010).

**Coagulase Test**
Three test tubes were labeled: 'T' for the test organism, 'P' for the positive control (Staphylococcus aureus), and 'N' for the negative control (sterile broth). To each tube, 0.2 ml of serum was added. Tube 'T' received 0.8 ml of the test organism broth culture, tube 'P'

received 0.8 ml of the Staphylococcus aureus broth culture, and tube 'N' received 0.8 ml of sterile broth. The tubes were gently mixed and incubated at 37°C. After 2 hours, they were examined for clot formation (Cheesbrough, 2010).

**Haemolysis Activity**

The hemolysis activity of *Staphylococcus aureus* isolates was tested using blood agar. Blood Agar Base was autoclaved at 121°C for 15 minutes. After cooling to 45°C–50°C, sterile sheep blood (17% defibrinated) was added and mixed. About 20 ml of the medium was poured into Petri dishes and allowed to dry. A colony of *Staphylococcus aureus* was streaked onto the plate using an inoculating loop and incubated at 37°C for 24 hours. The plates were then examined for haemolysis (Cheesbrough, 2010).

**Mannitol Fermentation**

Mannitol Salt Agar was prepared according to the manufacturer's instructions. The isolates were subcultured onto the medium and incubated for 24 hours at 37°C. After overnight incubation, the plates were observed for mannitol fermentation. The presence of yellow colonies indicated positive mannitol fermentation, while pink colonies indicated no mannitol fermentation by the organisms (Cheesbrough, 2010).

**Antibiotics Susceptibility Testing**

**Preparation of McFarland Standard**

To prepare the turbidity standard (1% sulfuric sulfate suspension), 1 ml of concentrated sulfuric acid ($H_2SO_4$) was diluted in 99 ml of water. A 1% solution of barium chloride was made by dissolving it in 500 ml of distilled water. Then, 0.6 ml of barium chloride solution was mixed with 99.4 ml of sulfuric acid to form the turbid solution, which matches the 0.5 McFarland standard (Cheesbrough, 2010).

**Susceptibility Testing:**

A pure culture of isolated staphylococci was emulsified in sterile saline until the turbidity matched the 0.5 McFarland standard. A sterile swab was dipped into the solution, excess fluid removed, and the swab streaked evenly on a sterile Muller-Hinton agar plate. Antibiotic disks (Oxoid UK) including Gentamycin (10 µg), Ciprofloxacin (10 µg), Erythromycin (15 µg), Tetracycline (30 µg), Cotrimoxazole (25 µg), and Clindamycin (2 µg) were placed on the agar surface using sterilized forceps. Plates were inverted and incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured and compared to CLSI standards (2018).

**Screening of Methicillin Resistant *Staphylococcus aureus***

Methicillin resistant *Staphylococcus aureus* was phenotypically screened using a 30µg Cefoxitin antibiotic disk following a modified Kirby–Bauer disk diffusion technique.

**Cefoxitin Disc Diffusion**

Cefoxitin disk (30µg) susceptibility testing was performed according to the Clinical Laboratory Standards Institute (CLSI, 2018). Briefly, a bacteria suspension adjusting to 0.5 McFarland was inoculated onto Muller – Hinton agar. A filter paper disk containing 30µg Cefoxitin was placed on the inoculated Muller – Hinton agar. The inoculated plates were incubated at 35°C for 24 hours and the zones of inhibition was measured (CLSI, 2018).

**Phenotypic Screening for Inducible Clindamycin Resistance**
Inducible clindamycin resistance in staphylococci was detected using the disk diffusion method with Clindamycin (2 µg) and Erythromycin (15 µg) disks.

The D-test was conducted by spreading the test staphylococci on Muller-Hinton agar, placing the Clindamycin and E erythromycin disks about 15 mm apart, and incubating at 35°C for 24 hours. A D-shaped zone of inhibition indicated a positive D-test, signifying that Erythromycin induced Clindamycin resistance (CLSI, 2018).

**Statistical Analysis**
The data generated in this study was analyzed using the Statistical Package for Social Sciences (SPSS) for windows version 25.0 used for statistical analysis and data interpretation. All the distribution was expressed in frequency and percentages and compared using Chi-square ($\chi^2$) test. All comparisons were Conducted using a 5% significance level at 95% confidence level.

**RESULTS**
From the 150 samples collected, 89 (59.3%) were from MMSH, and 61 (40.7%) were from MAWTH. Of 150 isolates obtained 149 (99.3%) were confirmed to be *Staphylococcus aureus* and the remaining 1 (0.7%) was *Staphylococcus epidermis* (Table 1). The majority of the isolates, 87 (58.0%) were obtained from wound swabs, followed by 28 (18.7%) and 19 (12.7%) obtained from urine and sputum samples, respectively (Figure 1).

Table 2 showed the distribution of the positive MRSA and D-test among the isolated *Staphylococcus* spp. Fifty-nine (39.3%) of the isolated were MRSA positive and 18 (12.0%) were confirmed to be D-test positive. Given an overall MRSA prevalence of 39.3% and a D-test prevalence of 12.0%.

Fourteen (9.3%) of the isolates were both MRSA and D-test positive, 4 (2.7%) were only D-test positive but MRSA negative and 45 (30.0%) were MRSA positive but D-test negative (Table 3).

Among the antibiotics tested, clindamycin showed the highest sensitivity (66.0%) among the isolated Staphylococcal strains, followed by gentamycin (50.0%). The highest resistance rate (62.0%) was found with cotrimoxazole, followed by tetracycline (47.0%) and ciprofloxacin (46.0%) (Table 4).

Highest prevalence of 18.2% (2/11) inducible clindamycin resistance was found among participants aged ≥70 years followed by 13.2% (9/68) and 10.9% (5/46) among participants aged 10 – 29 and 30 – 49 years, respectively. The participants in age range <10 years had the least prevalence of 0 (0/5) inducible clindamycin resistance. Female participants had the highest prevalence of 14.6% (13/89) inducible clindamycin resistance when compared to 8.2% (5/61) male (Table 5).

**Table 1: Distribution of the Isolated and their Source**

| Location | Frequency | Percentages |
|---|---|---|
| MAWTH | 61 | 40.7 |
| MMSH | 89 | 59.3 |
| Total | 150 | 100.0 |
| **Total** | **150** | **100.0** |
| **Isolated Organisms** | | |
| *Staphylococcus epidermis* | 1 | 0.7 |
| *Staphylococcus aureus* | 149 | 99.3 |
| **Total** | **150** | **100.0** |

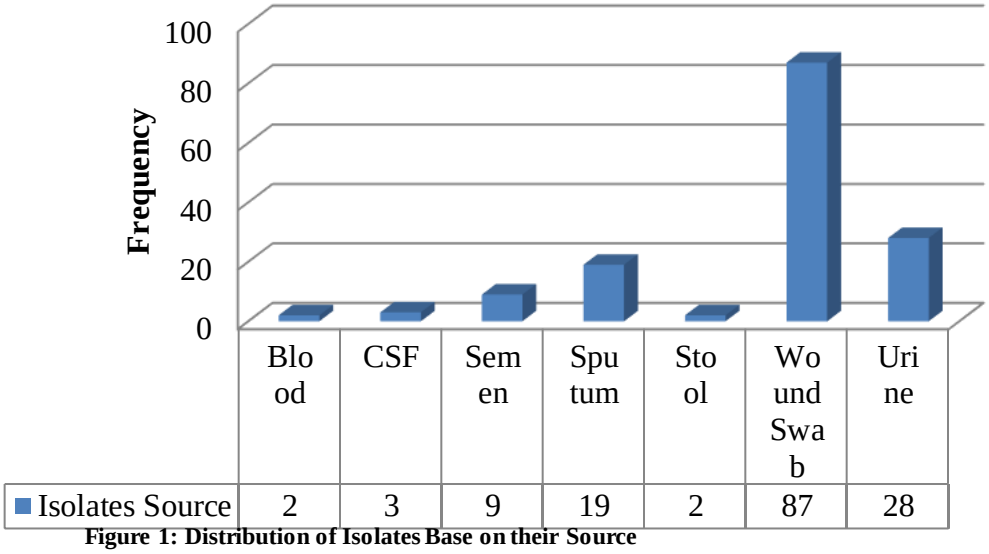


**Figure 1: Distribution of Isolates Base on their Source**

**Table 2: Distribution of the Positive MRSA D-Test Isolates**

| | Frequency | Percentages |
|---|---|---|
| **MRSA** | | |
| Positive | 59 | 39.3 |
| Negative | 91 | 60.7 |
| **Total** | **150** | **100.0** |
| **D-TEST** | | |
| Positive | 18 | 12.0 |
| Negative | 132 | 88.0 |
| **Total** | **150** | **100.0** |

**Table 3: Comparison between MRSA and D-Test Staphylococcus Isolates**

| D-Test | MRSA | | Total |
|---|---|---|---|
| | Positive n (%) | Negative n (%) | |
| Positive | 14 (9.3) | 4 (2.7) | 18 (12.0) |
| Negative | 45 (30.0) | 87 (58.0) | 132 (88.0) |
| **Total** | **59 (39.3)** | **91 (60.7)** | **150 (100.0)** |

P=0.001

**Table 4: Antibiotics Susceptibility Pattern of the Isolated Staphylococcal using Conventional Antibiotics**

| Antibiotics (µg) | Sensitivity Pattern | | |
|---|---|---|---|
| | Sensitivity n (%) | Intermediate n (%) | Resistance n (%) |
| Ciprofloxacin (CIP 10µg) | 73 (48.0) | 9 (6.0) | 69 (46.0) |
| Erythromycin (ERY 15µg) | 72 (48.0) | 6 (4.0) | 72 (48.0) |
| Tetracycline (TET 30µg) | 62 (41.0) | 18 (12.0) | 71 (47.0) |
| Cotrimoxazole (SXT 25µg) | 56 (37.3) | 1 (0.7) | 93 (62.0) |
| Gentamycin (CN 10µg) | 75 (50.0) | 0 (0.0) | 75 (50.0) |
| Clindamycin (DA 2µg) | 99 (66.0) | 3 (2.0) | 48 (32.0) |

**Table 5: Comparison between Demographic Distribution of the Study Participants and D-Test Staphylococcus Isolates**

| Demographic Parameters | No. Tested | D-Test | | *P - value* |
|---|---|---|---|---|
| | | Positive n (%) | Negative n (%) | |
| **Age Group (Years)** | | | | |
| <10 | 5 (3.3) | 0 (0.0) | 5 (100.0) | 0.860 |
| 10 – 29 | 68 (45.3) | 9 (13.2) | 59 (86.8) | |
| 30 – 49 | 46 (30.7) | 5 (10.9) | 41 (89.1) | |
| 50 – 69 | 20 (13.3) | 2 (10.0) | 18 (80.0) | |
| ≥70 | 11 (7.3) | 2 (18.2) | 9 (81.8) | |
| **Total** | **150 (100.0)** | 18 (12.0) | 132 (88.0) | |
| **Sex** | | | | |
| Male | 61 (40.7) | 5 (8.2) | 56 (91.8) | 0.177 |
| Female | 89 (59.3) | 13 (14.6) | 76 (85.4) | |
| **Total** | **150 (100.0)** | 18 (12.0) | 132 (88.0) | |

## DISCUSSION

The overall prevalence rate of MRSA detected using the disk diffusion method was 39.3%. This rate is higher than the 20.6% and 21.5% prevalence rates reported by Idris *et al.* (2019) among patients with septicemia and by Idris *et al.* (2018) from clinical isolates respectively. In contrast, Okoye *et al.* (2022) reported 27.6% prevalence in Enugu, Southern Nigeria, while Sadauki *et al.* (2022) documented a lower prevalence of 2.8% from nasal samples at the Infectious Disease Hospital in Kano. However, the prevalence observed in this study is lower than the 46.9% and 60.3% rates reported by Adelza *et al.* (2020) and Hussaini *et al.* (2018), respectively, both from Sokoto State.

Kishk *et al.* (2020) emphasized that routine in vitro tests for clindamycin susceptibility might fail to detect inducible clindamycin resistance when macrolide-lincosamide-streptogramin B ($MLS_B$) resistance is present due to erm genes. Identifying inducible clindamycin resistance associated with the $MLS_B$ phenotype is essential for guiding appropriate antibiotic therapy and preventing the spread of resistant bacteria. Therefore, the D-test is recommended as a valuable tool in routine testing to accurately identify this resistance mechanism (Hamza *et al.*, 2023).

In this study, 12.0% of the Staphylococcal isolates exhibited true inducible clindamycin resistance, indicating that patients infected with these isolates cannot be effectively treated with clindamycin. This finding aligns with previous studies in Nigeria, such as those by Ifediora *et al.* (2019) in Abia State which reported similar prevalence rates of 12.1%.

Other studies, both within and outside Nigeria, have reported slightly higher rates of inducible clindamycin resistance, with prevalence ranging from 15.5% to 17.7% in research by Kavitha (2020), Jahanbakhshi *et al.* (2023), and Okojokwu *et al.* (2018). Additionally, significantly higher incidences of 37.5% and 25.0% were reported by Lall and Sahni (2014) in India, and by Hamza *et al.* (2023) in Katsina State and Abdullahi *et al.* (2022) in Kaduna State, Nigeria, respectively. These findings underscore the need to monitor and understand the occurrence of inducible clindamycin resistance among Staphylococcal isolates to inform effective treatment strategies and combat the spread of antibiotic resistance.

Understanding antibiotic susceptibility patterns is crucial for clinicians in selecting the most effective treatment regimens for bacterial infections. This knowledge helps anticipate potential therapeutic challenges and enables informed decision-making to optimize patient outcomes. By identifying the antibiotics most effective against Staphylococcal isolates,

healthcare professionals can tailor treatment plans to address specific resistance profiles, ultimately enhancing the efficacy of antibiotic therapy.

The findings from this study on antibiotic susceptibility patterns of Staphylococcal isolates are particularly significant for bacterial treatment. The study showed that 66.0% of the isolates were sensitive to clindamycin, suggesting it as a viable treatment option for Staphylococcal infections. Additionally, 50.0% of the isolates were susceptible to gentamicin, and 48.0% to both ciprofloxacin and erythromycin, indicating that these antibiotics could also be considered in managing such infections.

These sensitivity results align with those from previous studies, including those by Idris *et al.* (2019), Ifediora *et al.* (2019), Idris *et al.* (2018), and Hussaini *et al.* (2018), which collectively identified ciprofloxacin, gentamicin, clindamycin, and erythromycin as optimal choices for treating Staphylococcal infections. The consistency across multiple studies highlights the effectiveness of these antibiotics, providing valuable insights for clinicians in selecting appropriate treatment regimens. The convergence of results reinforces the importance of these antibiotics in managing Staphylococcal infections, giving healthcare providers greater confidence in their treatment decisions based on a robust evidence base.

## CONCLUSION

The study revealed a concerning prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) at 39.3% and 12.0% inducible clindamycin resistance among *Staphylococcus aureus* isolates. These findings highlight the challenges posed by antibiotic-resistant strains of *Staphylococcus aureus*, which can complicate treatment and increase healthcare costs. In terms of effective antibiotics for treating Staphylococcus bacterial infections, the study identified several options. Clindamycin, gentamicin, ciprofloxacin, and erythromycin were found to be among the most effective antibiotics for treating infections caused by Staphylococcus bacteria. This information is crucial for healthcare providers when selecting appropriate antibiotic treatments, especially in the context of increasing antibiotic resistance.

## RECOMMENDATIONS

Based on the findings of this study, it was recommend the implementation and strengthening of antibiotic stewardship programs in the study sites to optimize antibiotic use, including clindamycin, and prevent the development and spread of antibiotic resistance. Routine testing for inducible clindamycin resistance in *Staphylococcus aureus* isolates, particularly methicillin-resistant strains, is also essential to guide appropriate antibiotic therapy and improve patient outcomes.

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