Inducible Clindamycin Resistance in *Staphylococcus aureus* Isolated from Palms of Poultry Workers in Jos, Plateau State, Nigeria


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**Abstract** | **Background:** Clindamycin is used for the treatment of infections attributed to macrolide (erythromycin) resistant *Staphylococcus aureus*; particularly infections of skin and soft tissues. Therapy for staphylococcal infections may be complicated by the possibility of inducible macrolide–lincosamide–streptogramin B resistance (iMLS₆₅). **Objective:** This study was carried out to assess the prevalence of phenotypic expression of inducible clindamycin resistance of *Staphylococcus aureus* isolated from palms of poultry workers in Jos, Plateau State, Nigeria. **Methods:** A total of 186 *Staphylococcus aureus* were isolated and identified by conventional methods and subjected to antibiotic susceptibility testing by Kirby–Bauer disk diffusion method. Double disc approximation test (D-test) was used to investigate inducible and constitutive MLS₅ resistant phenotype. **Results:** From 186 *S. aureus* isolates, 113 (60.8%) were erythromycin resistant and 20 (10.8%) were clindamycin resistant. Most of the isolates 155 (83.3%) were methicillin-sensitive *S. aureus* (MSSA) while 31 (16.7%) were resistant to methicillin (MRSA). Out of the 186 isolates, 33 (17.7%) were iMLS₆₅ phenotype (D-test positive), 20 (10.8%) were constitutively resistant (cMLS₆₅ phenotype) and 60 (32.3%) were methicillin-sensitive (MS) phenotype (D-test negative). The incidence of constitutive and inducible clindamycin resistant phenotypes were higher in MRSA than MSSA. On the other hand, the incidence of MS phenotype was higher in MSSA than in MRSA. **Conclusion:** The study revealed that 17.7% of *S. aureus* were inducible clindamycin resistant which could have been misidentified as clindamycin susceptible by Kirby–Bauer disk diffusion method. The study also showed the importance of D-test in detecting inducible clindamycin resistance in *S. aureus*.

**Keywords** | Methicillin resistant, *Staphylococci*, Clindamycin, Inducible resistance, Constitutive resistance, D-test

**INTRODUCTION**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most widespread nosocomial pathogens (Harbath et al., 2005). The bacteria can be categorized as hospital-associated MRSA (HA-MRSA), community-associated MRSA (HA-MRSA) and livestock-associated MRSA (LA-MRSA) (Dahms et al., 2014). Close contacts to farm workers, especially family members, are at higher risk of acquiring MRSA (Dahms et al., 2014). Erythromycin (a macrolide) and clindamycin (a lincosamide) represent two distinct classes of antimicrobial agents of the MLS₅ family. Both of them bind to the 50S ribosomal subunit thereby inhibiting protein synthesis (Yoon et al., 2008). The resistance to these two drugs can be mediated by msrA gene (MS phenotype) conferring the efflux mechanism (Ciraj et al., 2009; Deotale et al., 2010) or via the erm gene which encodes for the enzyme producing inducible or constitutive resistance to MLS₅ (Laclercq, 2002; Yoon et al., 2008). The resistance is constitutive (cMLS₅) when
R-methylase is produced and inducible ($iMLS_b$) when methylase is produced only in the presence of an inducing agent.

To the best of our knowledge, no previous data on prevalence of drug resistance to erythromycin-clindamycin are available in Plateau State, Nigeria. This work therefore was done to close or address this knowledge gap. The aim of this work is to screen for LA-MRSA in poultry workers and to detect the prevalence of inducible clindamycin resistance among the $S.\ aureus$ isolated from poultry workers.

**MATERIALS AND METHODS**

**Study Area**

This study was carried out in Jos (Jos South, North and East Local Government Areas) capital of Plateau State, North Central region of Nigeria. With about, nine hundred thousand (900,000) residents (NPC, 2006). It has a latitude of 9°56’N and longitude 8°53’E with monthly mean temperature of 21° – 25°C and 179km (111miles) from Abuja, the nation’s Federal Capital Territory (AGIS, 2010).

The study was a prospective study conducted during a period of 8 months (August 2015 to March 2016). A total of 186 $Staphylococcus aureus$isolated from palms of poultry workers were used in the study. $Staphylococcus aureus$ isolates were identified by standard biochemical techniques (Colle et al., 2006). Antimicrobial Susceptibility Testing (AST) was carried out for the coagulase positive $S.\ aureus$ isolates using Kirby-Bauer disc diffusion method and interpreted as recommended by Clinical and Laboratory Standards Institute guidelines (CLSI, 2013). Antibiotic discs used were penicillin discs (10 units), cotrimoxazole discs (23.75/1.25 µg), cefoxitin discs (30 µg), cefuroxime discs (30 µg), gentamycin (10 µg), erythromycin discs (15 µg) and clindamycin (2 µg).

Isolates that were resistant to cefoxitin (30 µg) disc with zone of inhibition of inhibition ≤ 22 mm were taken to be methicillin resistant $S.\ aureus$ (MRSA) while those with zones of inhibition were considered methicillin sensitive $S.\ aureus$ (MSSA). Methicillin sensitive coagulase negative $S.\ aureus$ (MRCoNS) were identified. All isolates were subjected to inducible clindamycin resistance testing by CLSI recommended D-test on Mueller Hinton agar by keeping erythromycin (15 µg) disc and clindamycin disc (2 µg) disc at 15 mm apart (edge to edge) (CLSI, 2013). Blunting of the circular zone of inhibition around clindamycin disc towards erythromycin disc indicated the presence of $iMLS_b$ resistance and was reported as resistance to clindamycin.

Following overnight incubation at 37°C, three different phenotypes were appreciated and interpreted as follows:

1. Constitutive $MLS_b$ phenotype: $iMLS_b$ $S.\ aureus$ isolates which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size≤14 mm) with circular shape zone of inhibition around clindamycin.

2. Inducible $MLS_b$ phenotype: $iMLS_b$ $S.\ aureus$ isolates which showed resistance to erythromycin (zone sizes≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (D test positive).

3. Methicillin-sensitive (MS) phenotype: $S.\ aureus$ isolates exhibiting resistance to erythromycin (zone size ≤13 mm), while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin (no D zone i.e. D test negative).

**Statistical Analysis**

Statistical analysis was performed using Stata version 13, and P-values of ≤ 0.05 were considered statistically significant.

**RESULTS**

One hundred and eighty-six (186) staphylococcal isolates were obtained from palms of poultry workers, out of which 133 were resistant to erythromycin (87 methicillin susceptible $S.\ aureus$ and 26 methicillin resistant $S.\ aureus$) while 20 $S.\ aureus$ (15 methicillin susceptible $S.\ aureus$ and 5 methicillin resistant $S.\ aureus$) were resistant to clindamycin (Table 1). One hundred and fifty-five [155(83.3%)] were susceptible to methicillin (MSSA) while 31(16.7) were methicillin resistant (MRSA). One hundred and thirteen [133(60.8%)] were found to be resistant to erythromycin; 20(10.8%) isolates were resistant to both erythromycin and clindamycin. Out of the 20 isolates, 15 isolates were methicillin sensitive $S.\ aureus$ (MSSA) representing 9.7% of the MSSA while 5(16.1%) were methicillin resistant $S.\ aureus$ (MRSA). This suggests constitutive macrolide-lincosamide-streptogramin B ($MLS_b$) phenotype.

Table 3 depicts the relationship of D-test in relation to sex.
### Table 1: Resistance profile of *S. aureus* to erythromycin and clindamycin

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MSSA (n = 155)</th>
<th>MRSA (n = 31)</th>
<th>Total (n = 186)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin (15 µg)</td>
<td>87 (56.1%)</td>
<td>26 (83.9%)</td>
<td>113 (60.8%)</td>
</tr>
<tr>
<td>Clindamycin (2 µg)</td>
<td>15 (9.7%)</td>
<td>5 (16.1%)</td>
<td>20 (10.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>102 (65.8%)</td>
<td>31 (100.0%)</td>
<td>133 (71.5%)</td>
</tr>
</tbody>
</table>

**MSSA** = Methicillin sensitive *Staphylococcus aureus*, **MRSA** = Methicillin resistant *Staphylococcus aureus*

### Table 2: Resistance phenotype of *Staphylococcus aureus*

<table>
<thead>
<tr>
<th></th>
<th>Total isolates</th>
<th>Constitutive MLS&lt;sub&gt;B&lt;/sub&gt; resistance (ERY-R, CLI-R)</th>
<th>Inducible MLS&lt;sub&gt;B&lt;/sub&gt; resistance (ERY-R, CLI-S, D+)</th>
<th>MS phenotype (ERY-R, CLI-S, D–)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>155 (83.3%)</td>
<td>15 (9.7%)</td>
<td>20 (12.9%)</td>
<td>52 (33.5%)</td>
</tr>
<tr>
<td>MRSA</td>
<td>31 (16.7%)</td>
<td>5 (16.1%)</td>
<td>13 (41.9%)</td>
<td>8 (25.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>186 (100.0%)</td>
<td>20 (10.8%)</td>
<td>33 (17.7%)</td>
<td>60 (32.3%)</td>
</tr>
</tbody>
</table>

ERY-R: Erythromycin resistant (diameter of zone of inhibition ≤13mm); CLI-R: Clindamycin resistant (diameter of zone of inhibition ≤14mm); CLI-S: Clindamycin susceptible (diameter of zone of inhibition ≥21mm); MS: Methicillin sensitive; D+: D-shaped clear zone around CLI disc proximal to ERY disc; D–: Circular clear zone around CLI only; MLS<sub>B</sub>: Macrolide-lincomamide-streptogramin B.

### Table 3: Chi square comparison of D-test positive and D-test negative *S. aureus*

<table>
<thead>
<tr>
<th>D-test</th>
<th>Isolates, n = 186</th>
<th>Sex, n = 186</th>
<th>MRSA, n = 31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSSA (%)</td>
<td>MRSA (%)</td>
<td>Male (%)</td>
</tr>
<tr>
<td><strong>D+</strong></td>
<td>20 (60.6)</td>
<td>13 (39.4)</td>
<td>13 (39.4)</td>
</tr>
<tr>
<td><strong>D–</strong></td>
<td>135 (88.2)</td>
<td>18 (11.8)</td>
<td>59 (38.6)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>155 (83.3)</td>
<td>31 (16.7)</td>
<td>72 (38.7)</td>
</tr>
<tr>
<td>(\chi^2)</td>
<td>14.920</td>
<td>0.008</td>
<td>0.217</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001**</td>
<td>0.929</td>
<td>0.641</td>
</tr>
<tr>
<td>Association</td>
<td>Highly significant</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

There was no statistically significant association (\(\chi^2 = 0.008; p = 0.929\)) between the response of the isolates to D-test and sex. Similarly, there was no association (\(\chi^2 = 0.217; p = 0.641\)) between the response of MRSA to D-test and sex. On the other hand, there was statistically significant association (\(\chi^2 = 14.920; p < 0.001\)) between D-test and type of *S. aureus*. Methicillin-sensitive *S. aureus* had higher D-test negative [135(88.2%)] isolates than MRSA [18(11.8%)]. In the same vain, more MSSA [20(60.6)] were positive than MRSA [13(39.4%)].

### DISCUSSION

This study established the existence of methicillin resistant *Staphylococcus aureus* on the hands of poultry farmers in Jos metropolis. Methicillin-resistant *Staphylococcus aureus* contamination of poultry products increases the emergence of antimicrobial resistance to humans. Poultry products are considered one of the main sources of spread of MRSA in humans. Commercial flocks, live bird markets, poultry litter, poultry slaughter houses and manure also play vital roles in propagation of resistant bacteria strain(s). This emphasizes the possibility of transmission between poultry birds (animals) and human and possibly from contact with pets at home. *Staphylococcus aureus* is one of the major pathogens that cause bacteremia and/or nosocomial infections. Antimicrobial resistance is an increasing problem in *S. aureus* infections all over the world (Tekin et al., 2013). The emergence of MRSA isolates led to difficulties for the treatment of infections caused by this microorganism. At the moment, up to 95% of clinical staphylococcal isolates are resistant to penicillin (Campanile et al., 2001; Sakoulas and Moellering, 2008). In the present study, methicillin resistance of *S. aureus* was found to be 16.7%. The difference in the prevalence of MRSA in different locations indicated that local antimicrobial testing has a significant role in empirical therapeutic decision making (Tekin et al., 2013). It is our opinion that 16.7% prevalence of MRSA in the poultry workers’ palm is high considering that these people were apparently healthy. It also suggests that beta-lactam antibiotics are inefficient agents for *S. aureus* infections hence it will be necessary to employ the use of different antibiotics for the treatment of infections caused by *S. aureus*.

Clindamycin is an alternative antibiotic for patients who are allergic to beta-lactam or who suffer from infections caused by MRSA. Clindamycin is indicated in the treat-
ment of skin and soft-tissue infections caused by *Staphylococcus* species (Drinkovic et al., 2001; Nwokah and Abbey, 2016). Treatment failure can result when clindamycin or any non-inducer macrolide is used to treat infection caused by staphylococcal strain carrying inducible *erm* gene (Drinkovic et al., 2001). Therefore, *in vitro* test for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to *erm* genes which eventually results in treatment failure, thus the need to routinely screen for or detect such resistance by the double disk approximation test (D-test).

In this study, 60.8% of staphylococcal isolates were erythromycin resistant. Thirty-three (33 i.e. 17.7%) of the erythromycin resistant *S. aureus* isolates showed inducible clindamycin resistance. Some investigators have reported a higher incidence of iMLS$_b$ resistance while others indicated a lower incidence (Kumurya, 2015; Nwokah and Abbey, 2016). We observed, in our study, a similar incidence of iMLS$_b$ (17.7%) resistance among *S. aureus* and iMLS$_b$ (10.8%), even though a few others reported variable results. This difference or variability could be attributed to difference in geographical location, methicillin susceptibility of the *S. aureus* isolates and age group of the study subjects (Mohanansoundaram, 2011). The higher percentage of inducible clindamycin resistance in MRSA isolates (41.9%) as compared to MSSA (12.9%) is in concordance with various studies that reported the prevalence of erythromycin-induced clindamycin resistance (Mohanansoundaram, 2011). We also observed that the MS phenotype was higher in the MSSA (33.5%) than MRSA (25.8%) which was in agreement with the findings of previously reported findings (Fiebelkorn et al., 2003; Fokas et al., 2005).

**CONCLUSION**

On account of emergence of resistance to antimicrobial agents among *Staphylococcus aureus*, the accurate antibiotic susceptibility data of the infecting bacteria is pertinent for making informed therapeutic decisions. On the whole, the inducible clindamycin resistant isolates obtained in our study was 17.7%. If D-test was not performed, those isolates would have been misidentified as being clindamycin susceptible thereby leading to therapeutic failure. Hand hygiene by poultry workers is therefore advocated to help reduce transfer of iMLS$_b$ *Staphylococcus aureus* from one person to the other.

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