OPEN ACCESS
Journal of Infection and Molecular Biology

Research Article

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 (*i*MLS$_B$). **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 MLS$_B$ 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 *i*MLS$_B$ phenotype (D-test positive), 20 (10.8%) were constitutively resistant (*c*MLS$_B$ 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

Editor | Tahir Yaqub, University of Veterinary and Animal Sciences, Lahore, Pakistan.
Received | January 18, 2018; **Accepted** | February 11, 2018; **Published** | May 11, 2018
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Citation | Okojokwu OJ, Akpakpan EE, Azi HY, Abubakar BS, Anejo-Okopi JA (2018). Inducible clindamycin resistance in *staphylococcus aureus* isolated from palms of poultry workers in jos, plateau state, nigeria. J. Inf. Mol. Biol. 6(1): 11-15.
DOI | http://dx.doi.org/10.17582/journal.jimb/2018/6.1.11.15
ISSN (Online) | 2307-5465; **ISSN (Print)** | 2307-5716

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 MLS$_B$ family. Both of them bind to the50S 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 MLS$_B$ (Laclercq, 2002; Yoon et al., 2008). The resistance is constitutive (*c*MLS$_B$) 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*Staphylococcusaureus*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: cMLS$_B$*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 size≤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. aureua* (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 (cMLS$_B$) phenotype. Thirty-three [MSSA: 20(12.9%); MRSA: 13(41.9)] isolates were positive to D-test which indicated inducible macrolide-lincosamide-streptogramin B (iMLS$_B$) phenotype. Sixty-three [MSSA: 55(33.5%); MRSA: 8(25.8%)] isolates were negative to D-test indicating methicillin-sensitive (MS) phenotype. The isolates that were truly susceptible to clindamycin were 60. Relatively higher inducible and constitutive clindamycin resistance were observed in MRSA compared to MSSA (Table 2).

Table 3 depicts the relationship of D-test in relation to sex.

Table 1: Resistance profile of *S. aureus* to erythromycin and clindamycin

Antimicrobial agent	MSSA	MRSA	Total
	(n = 155)	(n = 31)	(n = 186)
Erythromycin (15 µg)	87 (56.1%)	26 (83.9%)	113 (60.8%)
Clindamycin (2 µg)	15 (9.7%)	5 (16.1%)	20 (10.8%)
Total	102 (65.8%)	31 (100.0)	133 (71.5%)

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

Table 2: Resistance phenotype of *Staphylococcus aureus*

	Total isolates	Constitutive MLS_B resistance (ERY-R, CLI-R)	Inducible MLS_B resistance (ERY-R, CLI-S, D+)	MS phenotype (ERY-R, CLI-S, D-)
MSSA	155(83.3%)	15(9.7%)	20 (12.9%)	52 (33.5%)
MRSA	31 (16.7%)	5 (16.1%)	13 (41.9%)	8 (25.8%)
Total	186 (100.0%)	20 (10.8)	33 (17.7)	60 (32.3%)

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_B: Macrolide-lincosamide-streptogramin B.

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

D-test	Isolates, n = 186		Sex, n = 186		MRSA, n = 31	
	MSSA (%)	MRSA (%)	Male (%)	Female (%)	Male (%)	Female (%)
D+	20 (60.6)	13 (39.4)	13 (39.4)	20 (60.6)	4 (30.8)	9 (69.2)
D-	135 (88.2)	18 (11.8)	59 (38.6)	94 (61.4)	7 (38.9)	11 (61.1)
Total	155 (83.3)	31 (16.7)	72 (38.7)	114 (61.3)	11 (35.5)	20 (64.5)
χ^2	14.920		0.008		0.217	
p-value	< 0.001**		0.929		0.641	
Association	Highly significant		Not significant		Not significant	

There was no statistically significant association (χ^2 = 0.008; p = 0.929) between the response of the isolates to D-test and sex. Similarly, there was not association (χ^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 (χ^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* in our communities 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 *i*MLS$_B$ resistance while others indicated a lower incidence (Kumurya, 2015; Nwokah and Abbey, 2016). We observed, in our study, a similar incidence of *i*MLS$_B$ (17.7%) resistance among *S. aureus* and *c*MLS$_B$ (10.8%), even though a few others reported variable results. This difference or vairablity 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 resisant 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 *i*MLS$_B$ *Staphylococcus aureus* from one person to the other.

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Microbiology, Faculty of Natural Sciences, University of Jos, Nigeria for providing bench space in their facilities where the work was carried out.

CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHORS CONTRIBUTION

Ocheme Julius Okojokwu conceived and designed the study, Ocheme Julius Okojokwu, Joseph AjeAnejo-Okopi, Hezekiah Yusuf Aziand Bashiru Shafa Abubakar performed the experiments; Ocheme Julius Okojokwu, Joseph AjeAnejo-Okopiand Bashiru Shafa Abubakar analyzed the data. Manuscript draft, reading, corrections and editing were done by Ocheme Julius Okojokwu, EdoamaEdetAkpakpan, Bashiru Shafa Abubakar and Hezekiah Yusuf Azi. All the authors consensually agreed to the final manuscript.

REFERENCES

- Abuja Geographic Information System (AGIS) (2010). Geographical location of Jos, Plateau State. Agis.fcta.gov.ng. Retrieved on 10th October, 2015.
- Campanile F, Cafiso V, Cascone C, Giannino V, Di Marco O, Stefani S (2001). Clonal diffusion and evolution of polymorphisms of *mec*A and Tn554 in methicillin-resistant *Staphylococcus aureus* in Italy. Le Infezioni in Medicina. 1:30 – 38.
- Ciraj AM, Vinod P, Sreejith G, Rajani K (2009). Inducible clindamycin resistance among clinical isolates of staphylococci. Indian J. Pathol. Microbiol. 52:49 – 51. https://doi.org/10.4103/0377-4929.44963
- Clinical and Laboratory Standards Institute CLSI (2013). Performance standards for antimicrobial susceptibility testing. 26th information supplement.
- Colle JG, Fraser AG, Marmion BP, Simmmons A, editors (2006). Mackie and McCartney, Practical Medical Microbiology. 14th ed. Amsterdam: Elsevier; 2006. https://doi.org/10.4081/mm.2006.3211
- Dahms C, Hubner N, Cuny C, Kramer A (2014). Occurrence of methicillin-resistant *Staphylococcus aureus* in farm workers and the livestock environment in Mecklenburg-Western Pomerania, Germany. Acta Veterinaria Scandinavica. 56:53 – 60. https://doi.org/10.1186/s13028-014-0053-3
- Deotale V, Mendiratta DK, Raut U, Narang P (2010). Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Indian J. Med. Microbiol. 28:124 – 126. https://doi.org/10.4103/0255-0857.62488
- Drinkovic D, Fuller ER, Shore KP, Holland DJ, Pegler E (2001). Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J. Antimicrob. Chemother. 48:315 - 316. https://doi.org/10.1093/jac/48.2.315
- Fiebelkorn KR, Crawford SA, McElmel ML, Jorgensen JH (2003). Practical disc diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase negative staphylococci. J. Clin. Microbiol. 41:4740 – 4744. https://doi.org/10.1128/JCM.41.10.4740-4744.2003
- Fokas S, Fokas S, Tsironi M, Kalkani M, Diony M (2005). Prevalence of inducible clindamycin resistance in macrolides resistant staphylococcal species. Clin. Microbiol. Infect. 11: 337 – 400. https://doi.org/10.1111/j.1469-

0691.2005.01101.x

- Harbath S, Francois P, Scranzel JM, Frankhanser-Rodriguez C, Hugonnet S, Koessleu T (2005). Community-associated methicillin-resistant *Staphylococcus aureus* Switzerland. Emerg. Infect. Dis. 11:962-965.
- Kumurya AS (2015). Detection of inducible clindamycin resistance among staphylococcal isolates from different clinical specimens in Northwestern Nigeria. Int. J. Prev. Med. Res. 1(2):35 – 39.
- Laclercq R (2002). Mechanisms of resistance to macrolides and lincosamides. Nature of resistance elements and their clinical implications. Clin. Infect. Dis. 34:482-92. https://doi.org/10.1086/324626
- Mohanasoundarm KM (2011). The prevalence of inducible clindamycin resistance among gram positive cocci from various clinical specimens. J. Clin. Diagn. Res. 5(1): 38-40.
- National Population Commission (NPC) (2006). National Census 2006.
- Nwokah EG, Abbey SD (2016). Inducible clindamycin resistance in *Staphylococcus aureus* isolates in Rivers State, Nigeria. Am. J. Clin. Expt. Med. 4(3):50 – 55. https://doi.org/10.11648/j.ajcem.20160403.13
- Sakoulas G, MoelleringJr RC (2008). Increasing antibiotic resistance among methicillin-resistant *Staphylococcus aureus* strains. Clin. Infect. Dis. 46:360 – 367. https://doi.org/10.1086/533592
- Tekin A, Dal T, Deveci O, Tekinn R, Almaca S, Dayan S (2013). Assessment of methicillin and clindamycin resistance patterns in *Staphylococcus aureus* isolated from a tertiary hospital in Turkey. Le Infezioni in Medicina 2:111-116.
- Yoon E, Kwon A, Min Y, Choi E (2008). Foggy D-shaped zone of inhibition in *Staphylococcus aureus*owing to a dual character of both inducible and constitutive resistance to macrolide lincosamidestreptogramin B. J. Antimicrob. Therapy. 61(3): 533-540. https://doi.org/10.1093/jac/dkn008