Preliminary Study on Constitutive-Clindamycin Resistant *Staphylococcus aureus* Isolates of Nosocomial Origin

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Abstract: The resistance to antimicrobial agents among Staphylococci in both the community and hospital environment is an increasing problem that requires proper monitoring and containment. Erythromycin (a macrolide), clindamycin (a lincosamide) and streptogramin B are important antibiotics used clinically for treating bacterial-related infections; but the emergence of constitutive- and inducible-resistance mechanisms (especially target-site modification in target bacteria) in some *S. aureus* strains puts the efficacy of these antimicrobials at risk. When the mechanism of resistance is constitutive, the bacteria produce rRNA methylase (that compromises the antimicrobial properties of the antibiotic *in vivo*); but in inducible resistance, rRNA methylase is only produced in the presence of an inducing agent such as erythromycin. In this preliminary study, we phenotypically detected the occurrence of constitutive-clindamycin resistance ($cMLS_B$) in 39 isolates of *S. aureus* of nosocomial origin. Antimicrobial susceptibility studies (antibiogram) were carried out using the Kirby-Bauer disk diffusion technique and $cMLS_B$ phenotypes was detected using 'D' test. Multiple antibiotic resistance indexes were calculated for $cMLS_B$ phenotypes. Our results of antibiogram shows that the *S. aureus* isolates were highly resistant to over 50 % of the tested antibiotics especially to cloxacillin (100 %), bacitracin (92.31 %), (53.85 %), mupirocin (82.05 %) and oxacillin (89.74 %). Reduced susceptibility of the *S. aureus* isolates was also observed in clindamycin (89.74 %), a weak inducer, and erythromycin (53.85 %), a potent inducing agent. A total of 6 (15.4 %) *S. aureus* isolates out of the 39 *S. aureus* isolates investigated phenotypically by 'D' test were confirmed as constitutive-clindamycin resistant phenotypes. With a MARI value of about 0.7 on average, the $cMLS_B$ phenotypes were multiply resistant to the tested antibiotics. Due to the importance of clindamycin (a weak inducer) as an alternative antibiotic for treating staphylococcal-related infections, it is important to preserve the efficacy of this antibiotic through proper detection of cMLSB phenotypes from clinical samples since *in vitro* susceptibility to clindamycin might lead to treatment failure especially if the invading bacterium is of constitutive-clindamycin resistance phenotype (that is producing rRNA methylase *in vivo*).

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1. Introduction

Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically on the human body. Although a member of the human microbiota, some pathogenic strains of *Staphylococcus* are major pathogens responsible for a variety of infections ranging from mild skin and soft tissue infections to life threatening conditions such as endocarditis, bacterial sepsis and pneumonia in which the organism is usually implicated (Brooks *et al*., 2010; Madigan *et al*., 2009). The clinical significance of pathogenic strains of *Staphylococcus aureus* has grown over the years, especially with the emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) strains that defy the antimicrobial efficacy of some antibiotics (Silva *et al*., 2016; Fomda *et al*., 2010). The notable resistance of pathogenic strains of *Staphylococcus* to some beta-lactams led to an increase in the use of macrolides (erythromycin), lincosamides (clindamycin) and streptogramin B, which correspond to the macrolide-lincosamide-streptogramin B (MLS_B) group of antibiotics. And this led to the rise of clindamycin resistant strains of *Staphylococcus* (which are either inducible- or constitutive-clindamycin resistant strains based on their level of enzyme expression) in the hospital environment as was previously reported (Silva *et al*., 2016; Bottega *et al*., 2014). Clindamycin is the

preferred antibiotic for the treatment of MRSA strains due to its excellent pharmacokinetic properties, such as optimum tissue penetration and abscess accumulation (Bottega *et al*., 2014). It is usually one of the most preferred and substitute antibiotic in the therapy of *Staphylococcus aureus* infection. However, there is increasing frequency of *Staphylococcus aureus* strains that are becoming resistant to the antimicrobial potency of this class of antibiotic, and these isolates are called clindamycin-resistant bacteria. MLS_B resistance may be constitutive ($cMLS_B$) or inducible ($iMLS_B$) in nature; and this is usually due to multiple resistance mechanisms that confer resistance to macrolides, lincosamide, and streptogramin B (MLS_B), thus making the bacterial organism to resist the antimicrobial action of MLS_B-group of antibiotics (Silva *et al*., 2016; Ejikeugwu *et al*., 2018; Sasirekha *et al*., 2014). In $cMLS_B$ expression, the bacterium has the erythromycin ribosomal methylase (*erm*) gene and is resistant to erythromycin and clindamycin, but in $iMLS_B$, the *erm* gene requires an inducing agent such as erythromycin to induce resistance (Silva *et al*., 2016). The *erm* gene encode enzymes that confer constitutive- or inducible-resistance to MLS_B agents through the methylation of the 23S rRNA of the target bacterium, and this inhibits or reduces the binding of the MLS_B agents to the ribosome of the bacteria, thereby causing either inducible- or constitutive-clindamycin resistance (Sasirekha *et al*., 2014; Silva *et al*., 2016; Bottega *et al*., 2014). Therapeutic failure following the usage of MLS_B-group of antibiotics for treating *Staphylococcus aureus* infection may persist in this part of the world – since constitutive- or inducible-clindamycin resistance is merely investigated in routine laboratory practice. And given that constitutive- or inducible-clindamycin resistance is ill-detected in this region, this study presumptively investigated the prevalence of constitutive-clindamycin resistance phenotypes among *S. aureus* isolates of nosocomial origin.

2. Material and Methods

Isolates: Non-duplicate clinical isolates of *Staphylococcus aureus* (n=39) were obtained from the culture collection unit of a Federal Teaching Hospital in Abakaliki, Ebonyi State, Nigeria. The isolates were re-identified as *S. aureus* isolates using standard microbiology techniques including colonial features on selective culture media, Gram staining, and biochemical tests (Cheesbrough, 2006).

Antimicrobial susceptibility testing: Kirby-Bauer disc diffusion method as recommend by Clinical and Laboratory Standard Institute (CLSI) was used to determine antimicrobial susceptibility profile of the *S. aureus* isolates. This was carried out on unsupplemented Mueller-Hinton (MH) agar plates inoculated with standardized test isolates. Antibiotic impregnated discs namely: clindamycin (2 µg), erythromycin (15 µg), cefoxitin (30 µg), cloxacillin (5 µg), mupirocin (5 µg), bacitracin (10 µg), oxacillin (1 µg) and gentamicin (10 µg) [Oxoid, UK] were placed on the MH agar plates using sterile forceps, and plates were incubated at 37°C for 18-24 h. Zones of inhibition around each disc were measured, recorded and interpreted using standard breakpoints of CLSI (CLSI 2011; Ejikeugwu *et al*., 2017).

Detection of constitutive-clindamycin resistance: Constitutive-clindamycin resistance was phenotypically evaluated using the D-test technique - in which erythromycin (15 µg) and clindamycin (2 µg) disk was used as the indicator antibiotics (Nwokah and Abbey, 2016; Ejikeugwu *et al*., 2018). Briefly, a 15-µg erythromycin disk was aseptically placed in proximity to a 2-µg clindamycin disk on MH agar plate that was previously inoculated with the test *S. aureus* isolate (adjusted to 0.5 McFarland turbidity standards). Susceptibility plates were incubated at 37°C for 18-24 h. Constitutive-clindamycin resistance was phenotypically inferred by flattening of the zone of inhibition around the clindamycin disk as well as to the erythromycin disk. More so, *S. aureus* isolates that showed resistance to clindamycin (zone size $\leq$ 14 mm) and erythromycin (zone size $\leq$ 13 mm) were also inferred to be constitutive-clindamycin resistant ($cMLS_B$) phenotypes (Nwokah and Abbey, 2016).

Multiple antibiotic resistance index (MARI): This was calculated only for *S. aureus* isolates positive for constitutive-clindamycin resistance, as was previously described (Ejikeugwu *et al.,* 2017; Ejikeugwu *et al*., 2018). MARI was calculated mathematically using the MARI formular as follows: MARI = $^{a}/_{b}$, where "a" is the number of antibiotics to which the resistant isolate was resistant to, and "b" is the total number of antibiotics to which the resistant isolate has been evaluated for.

3. Results

In this study, we presumptively investigated the frequency of constitutive-clindamycin resistance in *S. aureus* isolates of nosocomial origin; and we recruited a total of 39 non-duplicate *S. aureus* isolates from the culture collection unit of a tertiary hospital in Abakaliki, Nigeria for the study. Table 1 shows the frequency and distribution of the *S. aureus* isolates according to their specimen source. The susceptibility profile of the *S. aureus* isolates is shown in Table 2.

Table 1. Distribution of *S. aureus* isolates according to source

Source of Isolates	No of Samples	No (%) of *S. aureus* isolates
Urine	20	20 (50)
Blood	20	19 (47.5)
Total	40	39 (97.5)

Table 2. Antibiotic susceptibility profile

Antibiotics (µg)	Resistant (%)	Intermediate (%)	Susceptible (%)
FOX (30)	25(64.10)	0(0.00)	14(35.89)
B (10)	36(92.31)	0(0.00)	3(7.69)
E (15)	21(53.85)	13(33.33)	5(12.82)
OX (1)	35(89.74)	3(7.69)	1(2.56)
DA (2)	35(89.74)	2(5.13)	2(5.13)
CN (10)	13(33.33)	1(2.56)	25(64.10)
MUP (5)	32(82.05)	7(17.95)	0(0.00)
OB (5)	39(100.00)	0(0.00)	0(0.00)

Key: FOX-cefoxitin, B-bacitracin, E-erythromycin, OX-oxacillin, DA-clindamycin, CN-gentamicin, MUP-mupirocin, OB-cloxacillin

The *S. aureus* isolates showed varying levels of susceptibility to the tested antibiotics. Interestingly, *S. aureus* was highly resistant to cloxacillin (100 %) and bacitracin (92.31 %), which are important antibiotics used for the treatment of infections caused by Gram positive bacteria including *S. aureus*. Reduced susceptibility of the *S. aureus* isolates was also observed in cefoxitin (64.1 %), erythromycin (53.85 %), mupirocin (82.05 %), oxacillin (89.74 %) and clindamycin (89.74 %). However, some of the *S. aureus* isolates showed susceptibility to gentamicin (64.1 %), an aminoglycoside that targets Gram negative bacteria, and is bactericidal in action to both Gram positive and Gram negative bacteria (Table 2). Table 3 shows the frequency of *S. aureus* isolates that are constitutive-clindamycin phenotypes. Of the 39 isolates of *S. aureus* investigated phenotypically for constitutive-clindamycin resistance by the D-test technique in our study, only 6 (15.4 %) *S. aureus* isolates were confirmed phenotypically to be constitutive-clindamycin resistant isolates. These isolates were profoundly resistant to both clindamycin and erythromycin at varying degrees; and this is suggestive of constitutive-clindamycin resistance. Table 4 shows the result of the multiple antibiotic resistance indexes calculated for $cMLS_B$ *S. aureus* phenotypes. On average, the $cMLS_B$ phenotypes were found to be multiply resistant to the tested antibiotics at a MARI value of 0.7, showing that the *S. aureus* isolates that were positive for constitutive-clindamycin resistance ($cMLS_B$) were resistant to more than 50 % of the tested antibiotics.

Table 3. Frequency of constitutive-clindamycin *S. aureus* phenotypes

Isolate (n)	**$cMLS_B$ Positive n (%)**	**$cMLS_B$ Negative n (%)**
S. aureus (39)	6 (15.4)	33 (84.6)

Key: n-number of isolates; $cMLS_B$-constitutive-clindamycin resistance

Table 4. Multiple antibiotic resistance index (MARI)

Isolate	MARI Value	Antibiotics
B18	0.8	FOX,B,E,OX,CN,MUP, OB
B23	0.5	FOX, B,E,OX,DA
B25	0.8	FOX,B,E,OX,CN,MUP, OB
B28	0.8	FOX,B,E,OX,CN,MUP, OB
B30	0.5	FOX, B,E,OX,DA
B39	0.8	FOX,B,E,OX,CN,MUP, OB
Average MARI	0.7	-

Key: FOX-cefoxitin, B-bacitracin, E-erythromycin, OX-oxacillin, DA-clindamycin, CN-gentamicin, MUP-mupirocin, OB-cloxacillin

4. Discussions

Antimicrobial resistance in both the hospital and non-hospital environment is a global health phenomenon that is of public health interest since drug resistant bacteria despoil the antimicrobial efficacy of some available potent antimicrobials. Not to mention the least, some of these resistant phenotypes produce enzymes and harbour genes that allow them to become multidrug resistant in nature (Ejikeugwu *et al*., 2017); a phenomenon that is not too good for our antimicrobial armamentarium. Since the rate at which organisms develop resistance to some commonly used and available antibiotics is not at par with the rate at which novel antimicrobials are discovered and developed; it is therefore important for the medical community to always be on the lookout for these antibiotic resistant organisms especially by initiating proper protocol for their prompt detection, reporting and control in the hospital and non-hospital environment. In this study, *S. aureus* isolates of clinical origin showed reduced susceptibility to the tested antibiotics especially to cloxacillin (100 %), bacitracin (92.31 %), cefoxitin (64.1 %), erythromycin (53.85 %), mupirocin (82.05 %), oxacillin (89.74 %) and clindamycin (89.74 %). The high level of resistance of the *S. aureus* isolates to these antibiotics which are routinely used for the clinical management and/or treatment of infections caused by the organism – is suggestive of the resistance nature of these nosocomial isolates. More so, the high frequency of resistance recorded among the *S. aureus* isolates could be suggestive of their notable resistance to macrolides, lincosamides and streptogramin B (MLS_B) and other beta-lactam antibiotics. This could be because they possess certain genetic factors such as the *erm* genes that allow them to be multidrug resistant in nature (Silva *et al*., 2016; Ejikeugwu *et al*., 2018; Fomda *et al*., 2010; Sasirekha *et al*., 2014). The resistance of *S. aureus* to some commonly used antibiotics usually recommended for the treatment of infections caused by the organism has been previously reported (Nwokah and Abbey, 2016). In our study, only 6 (15. %) isolates of *S. aureus* were phenotypically confirmed to be constitutive-clindamycin resistant phenotypes. Elsewhere, the frequency of constitutive-clindamycin resistance phenotypes in *S. aureus* isolates from clinical samples have been previously reported (Seif *et al*., 2012; Bottega *et al*., 2014; Nwokah and Abbey, 2016). These isolates were also found to show varying levels of susceptibility/resistance to clindamycin and erythromycin, which were both the indicator antibiotics used for $cMLS_B$ detection and inducement. Constitutive-clindamycin resistance ($cMLS_B$) is common in *S. aureus* isolates that express the *erm* genes – which allow the bacterium to exhibit *in vivo and/or in vitro* resistance to erythromycin, clindamycin and other drugs of the MLS_B group of antibiotics (Bottega *et al*., 2014). As an important option in the treatment of staphylococci infections, clindamycin may gradually lose its efficacy due to the emergence of some mechanisms of resistance in *S. aureus* isolates (especially of clinical origin) that confer on the organism the ability to resist the antimicrobial onslaught of MLS_B group of antibiotics. In conclusion, the usage of MLS_B-group of antibiotics could result in treatment failure in cases where erythromycin and clindamycin are prescribed for the treatment of infections caused by *S. aureus* harbouring the *erm* genes. It is therefore important for Nigerian hospitals to be on the lookout for $cMLS_B$ phenotypes from clinical samples since routine antibiotic susceptibility testing is not sufficient enough to detect these resistance phenotypes. More so, further molecular characterization of our $cMLS_B$ phenotypes is required for the characterization of the molecular mechanism at play in our *S. aureus* isolates.

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References

1. Bottega A, Rodriguez M. de A, Carvalho F.A, Wagner T.F, Leal I.A.S, dos Santos S.O, Rampelotto R.F and Horner R (2014). Evaluation of constitutive and inducible resistance to clindamycin in clinical samples of *Staphylococcus aureus* from a tertiary hospital. *Revista da Sociedade Brasileira de Medicina Tropical*, 47(5):589-592.
2. Brooks G.F., Butel J.S and Morse S.A (2010). Medical Microbiology, 23rd edition. McGraw Hill Publishers. USA. Pp. 248-260.
3. Cheesbrough M (2006). District Laboratory Practice in Tropical Countries. 2nd edition. Cambridge University Press, UK. Pp. 178-187.
4. Clinical Laboratory Standard Institute, CLSI. Performance standards for antimicrobial disk susceptibility test. Fifteenth informational supplement, CLSI document M100-S15. 2011; CLSI, Wayne, PA. USA.
5. Ejikeugwu C., Iroha O., Amaechi C., Ugwu M., Eze P., Iroha C., Ogene L., Orinya C. (2017). Multiple Antibiotic Resistance, Antibiogram and phenotypic Detection of Metallo-Beta-lactamase (MBL) from *Escherichia coli* of poultry origin.

Journal of Apply Microbiology & Biotechnology, 1:4:5.

6. Ejikeugwu C, Nwezeagu F.J, Edeh C, Eze P (2018). Detection of constitutive- and inducible-clindamycin-resistance in clinical isolates of *Staphylococcus aureus* from a Federal Teaching Hospital in Abakaliki, Nigeria. *Journal of Bacteriology and Infectious Diseases*, 2(1):31-34.
7. Fomda, B. A., Peer, M. A., Zahoor, D., Thokar, M. A and Nasir, R. A (2010). Detection of Constitutive and Inducible Clindamycin Resistance. *J. Commun. Dis*. 42 (1):19-26.
8. Madigan M.T., Martinko J.M., Dunlap P.V and Clark D.P (2009). Brock Biology of Microorganisms, 12th edition. Pearson Benjamin Cummings Inc, USA.
9. Nwokah E.G and Abbey S.D (2016). Inducible-clindamycin resistance in *Staphylococcus aureus* isolates in Rivers state, Nigeria. *American Journal of Clinical and Experimental Medicine*, 4(3):50-55.
10. Sasirekha B, Usha M.S, Amruta J.A, Ankit S, Brinda N and Divya R (2014). Incidence of constitutive and inducible clindamycin resistance among hospital-acquired *Staphylococcus aureus*. *Biotech*, 4:85-89.
11. Seif N, Kahani N, Askari E, Mahdipour S and Naderi N.M (2012). Inducible clindamycin resistance in Staphylococcus aureus isolates recovered from Mashhad, Iran. *Iran Journal of Microbiology,* 4:82-86.
12. Silva, A. C., Silva, R. C. and Oliveira, S. R. (2016). Clindamycin microbial resistance in clinical isolates of *Staphylococcus sp*. derived from blood cultures of hospitalized patients. *J Bras Patol Med Lab*. 52 (3): 165-170.

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