

Detection of constitutive and inducible-clindamycin-resistance in clinical isolates of *Staphylococcus aureus* from a Federal Teaching Hospital in Abakaliki, Nigeria.

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Abstract

Infections caused by *Staphylococcus aureus* are usually treated with clindamycin. However, when treatment is directed against *S. aureus* isolates harbouring gene that mediate inducible clindamycin resistance, treatment failure is bound to occur. In this study, the occurrence of constitutive- and inducible-clindamycin resistance was phenotypically evaluated in clinical isolates of *Staphylococcus aureus* from a Federal Teaching Hospital in Abakaliki, Nigeria. A total of 39 non-duplicate clinical isolates of *S. aureus* were used in this study; and the isolates were subjected to antimicrobial susceptibility testing using cefoxitin (30 µg), bacitracin (10 µg), erythromycin (15 µg), oxacillin (1 µg), clindamycin (2 µg), gentamicin (10 µg), mupirocin (5 µg) and cloxacillin (5 µg). D-test was performed on all the *S. aureus* strains to detect constitutive- and inducible-clindamycin resistance (iMLS_B) phenotypes. The multiple antibiotic resistance nature of the iMLS_B phenotypes was calculated using multiple antibiotic resistance index (MARI) formula. Among the 39 clinical isolates of *S. aureus* studied, 76.9% (30/39) showed resistance to clindamycin, while 74.4% (29/39) showed resistance to erythromycin. Also, all the *S. aureus* isolates were completely resistant to cloxacillin and mupirocin. A total of 6 (15.4) *S. aureus* isolates showed inducible-clindamycin resistance (iMLS_B) while 20.5% *S. aureus* isolates (n=8) were confirmed constitutive-clindamycin resistant (cMLS_B) phenotypes by the D-test technique. On average, the *S. aureus* isolates that were positive for iMLS_B had MARI of 0.7; and this indicates a high level of multiple antibiotic resistance profile of the isolates. The result of this study has shown that cMLS_B and iMLS_B occur in clinical isolates of *S. aureus* from this part of the world. Further molecular characterization of the genetic factors responsible for cMLS_B and iMLS_B in clinical isolates of *S. aureus* is necessitated. Since routine antibiotic susceptibility studies cannot detect either cMLS_B or iMLS_B in clinical isolates of *S. aureus*, it is important for Nigerian hospital laboratories to include the D-test protocol in their practice.

Keywords: *Staphylococcus aureus*, cMLS_B, iMLS_B, D-test, Gram positive bacteria, Antimicrobial resistance

Accepted May 14, 2018

Introduction

Inducible or constitutive resistance to macrolide, lincosamide and streptogramin B (MLS_B) in clinical isolates of *Staphylococcus aureus* can result to clinical failure due to treatment with antibiotics in this family especially erythromycin and clindamycin. As opined by Sasirekha et al. [1], clindamycin is an important alternative antibiotic used for the treatment of infections caused by *S. aureus*. *S. aureus* is a Gram positive bacterium that is asporogenous in nature, and is implicated in both community and hospital-acquire infections globally [1-4]. The determination of the antimicrobial susceptibility pattern of clinical isolates of *S. aureus* especially to antibiotics in the MLS_B family is therefore crucial for the effective management of infections caused by the organism. Though the macrolide, lincosamide and streptogramin B (MLS_B) antibiotics have very similar antimicrobial activity targeted against the protein synthesis machinery of their target bacterial pathogen, they are chemically different antibiotics that are perfect alternatives to the other antibiotics to which *S. aureus* are least susceptible

[2,5,6]. However, their widespread and perhaps, irrational usage in either the community or hospital environment for the treatment of bacterial related diseases, has allowed *S. aureus* isolates resistant to this important class of antibiotics to emerge and spread. This situation is even worrisome in healthcare settings where inducible-clindamycin resistance is ill-detected or not detected at all in *S. aureus* isolates. The therapeutic failures due to the clinical usage of antibiotics in the MLS_B antibiotic family is increasingly being reported across the globe [1,2,5,7-9]. And this may reach alarming scenario in healthcare settings located in countries such as Nigeria – where the detection of such resistance phenotype may not be a routine medical practice in the hospital laboratory. The expression of resistance by clinical isolates of *S. aureus* to antibiotics in the MLS_B family may be constitutive (cMLS_B) or inducible (iMLS_B) in nature [1,4,5,9]. In constitutive resistance, rRNA methylase is always produced; whereas in inducible resistance, rRNA methylase is produced only in the presence of an inducing agent which can be any of the antibiotics in the MLS_B family such as erythromycin, a macrolide [7,9]. Clinical isolates of *S.*

aureus with constitutive resistance are resistant to clindamycin and erythromycin while those isolates with only inducible resistance are resistant to erythromycin but appear susceptible to clindamycin *in vitro* [8-10]. The use of clindamycin for therapy, especially in individuals with *Staphylococci* positive for inducible clindamycin resistance leads to the development of constitutive resistance, subsequently leading to therapeutic failure [6,11]. This study detected constitutive- and inducible-clindamycin resistance (MLSBI) among clinical isolates of *S. aureus* using the D-test technique.

Materials and Methods

Selection and re-identification of bacteria isolates

A total of 39 non-duplicate clinical isolates of *Staphylococcus aureus* were obtained from the culture collection unit of a Federal Teaching Hospital in Abakaliki, Ebonyi State, Nigeria for this study. All the isolates were re-identified to the species level using standard microbiology techniques including colonial morphology on growth media, coagulase test, catalase test and Gram staining technique [12].

Kirby-Bauer disk diffusion test

The standard antimicrobial susceptibility test was performed on each of the *S. aureus* isolates using the Kirby-Bauer disc diffusion method as recommend by Clinical and Laboratory Standard Institute (CLSI) on unsupplemented Mueller-Hinton (MH) agar plates inoculated with the standardized test isolates. The inoculated plates were allowed to stand for 10 to 15 minutes; and 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. The plates were incubated at 37°C for 24 hrs, and the zones of inhibition around each disc were measured, recorded and interpreted using standard zone size (breakpoints) of CLSI [13,14].

D-test

The detection of constitutive- and inducible-clindamycin resistance in the *S. aureus* isolates was phenotypically evaluated using the D-test technique - in which erythromycin (15 µg) and clindamycin (2 µg) disk was used [9]. D-test was performed on all isolates of *S. aureus* by placing a 15 µg erythromycin disk in proximity to a 2 µg clindamycin disk on MH agar plate that was previously inoculated with a staphylococcal isolate (adjusted to 0.5 McFarland turbidity standards). The susceptibility plates were then incubated overnight at 37°C. A flattening of the zone of inhibition around the clindamycin disk next to the erythromycin disk (producing a zone of inhibition shaped like the alphabet 'D') is considered a positive result. This result indicates that the erythromycin has induced clindamycin resistance. Constitutive-clindamycin resistance was however inferred in those *S. aureus* isolates that showed no inhibition zone size to clindamycin and erythromycin.

Multiple antibiotic resistance index (MARI)

Multiple antibiotic resistances were calculated for only isolates of *S. aureus* that showed inducible clindamycin resistance [14].

This was done 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.

Results

A total of 40 non-duplicate clinical isolates of *S. aureus* isolates from urine (n=20) and blood (n=20) samples were actually obtained and used for this study. However, after the re-characterization of the *S. aureus* isolates, a total of 39 isolates were phenotypically characterized and confirmed as *S. aureus* isolates; and these were used for the antimicrobial susceptibility testing and for the detection of constitutive- and inducible-clindamycin resistance using the D-test. The result of the antimicrobial susceptibility profile of the *S. aureus* isolates is shown in Table 1. All the isolates of *S. aureus* were highly resistant to the tested antibiotics especially cloxacillin (100%) and mupirocin (100%). Clindamycin and erythromycin also showed minimal antimicrobial activity against the *S. aureus* isolates that was used in this study (Table 1). Table 2 shows the result of the detection of constitutive and inducible-clindamycin resistance in the *S. aureus* isolates used in this study.

Only six (6) isolates of *S. aureus* out of the 39 isolates phenotypically screened for inducible clindamycin resistance (MLSB) phenotype in this study were found to be inducible clindamycin (iMLSB) positive – in which case the isolates showed susceptibility to clindamycin but with a 'D'-shaped zone of inhibition. However, 8 isolates of *S. aureus* were also confirmed positive for constitutive (cMLSB) clindamycin

Table 1. Antimicrobial susceptibility profile of 39 clinical isolates of *S. aureus*.

Antibiotics (µg)	Susceptible n (%)	Resistant n (%)
Cefoxitin (30)	17 (43.6)	22 (56.4)
Erythromycin (15)	10 (25.6)	29 (74.4)
Oxacillin (1)	4 (10.3)	35 (89.7)
Clindamycin (2)	9 (23.1)	30 (76.9)
Gentamicin (10)	29 (74.4)	10 (25.6)
Mupirocin (5)	0 (0)	39 (100)
Cloxacillin (5)	0 (0)	39 (100)
Bacitracin (10)	4 (10.3)	35 (89.7)

Table 2. Detection of constitutive- and inducible-clindamycin (MLSB) phenotypes.

Organism	Inducible (iMLS _B) resistance n (%)	Constitutive (cMLS _B) resistance n (%)
<i>S. aureus</i>	6 (15.4)	8 (20.5)

Table 3. Result of multiple antibiotic resistance index for iMLSB phenotypes.

Isolate No.	MARI	Antibiotics
B18	0.8	FOX, B, E, OX, DA, CN, MUP and OB
B23	0.5	FOX, B, E, OX and DA
B25	0.8	FOX, B, E, OX, DA, CN, MUP and OB
B28	0.8	FOX, B, E, OX, DA, CN, MUP and OB
B30	0.5	FOX, B, E, OX and DA
B39	0.8	FOX, B, E, OX, DA, CN, MUP and OB

KEY : FOX = Cefoxitin, B = Bacitracin, E = Erythromycin, OX = Oxacillin, DA = Clindamycin, CN = Gentamicin, MUP = Mupirocin and OB = Cloxacillin.

resistance – in which case they were found to be resistant to both clindamycin and erythromycin (Table 2). The result of multiple antibiotic resistance index of the inducible clindamycin resistant phenotypes is shown in Table 3.

Discussion and Conclusion

In isolates of *Staphylococcus aureus*, resistance to erythromycin (a macrolide) and clindamycin (a lincosamide) can occur through the methylation of their ribosomal target site on the target organism; and this is usually mediated by the *erm* genes harboured by the bacterium [1,7,9]. While both erythromycin and clindamycin are good antimicrobial agents that interfere with the protein synthesis of their target bacterium by binding to the 50S ribosomal subunits, the presence of inducible- and constitutive clindamycin resistance phenotypes in clinical isolates of *S. aureus* could render these antibiotics inefficacious for treatment. It is in view of this that this present study was targeted at detecting the possible occurrence of inducible-clindamycin- and constitutive clindamycin resistance in clinical isolates of *S. aureus* from a Federal Teaching Hospital in Abakaliki, Nigeria using the D-test technique since most hospital in Nigeria merely go beyond the routine antimicrobial susceptibility testing when a pathogen is recovered from clinical samples. The result of the antimicrobial susceptibility testing showed that the *S. aureus* isolates showed varying levels of susceptibility and resistance to the tested antibiotics. However, the *S. aureus* isolates completely showed reduced susceptibility to mupirocin (100 %) and cloxacillin (100 %) – which are used clinically to manage infections caused by *S. aureus*. More than 50% of the *S. aureus* isolates were also found to be highly resistant to clindamycin (76.9%), erythromycin (74.4%), oxacillin (89.7%) and cefoxitin (56.4%). The very high rates of the *S. aureus* clinical isolates to erythromycin, clindamycin, oxacillin and cefoxitin have been noted in previous studies in which *S. aureus* from both community and hospital samples was reported to be resistant to some commonly used antibiotics meant for the treatment of infections caused by the organism [1,2,5,10]. Previous studies show that the prevalence of inducible clindamycin resistance varies from one country to another. In this study, there was a 15.3% inducible-clindamycin (iMLSb) resistance phenotype (iMLSb) level amongst the *S. aureus* isolates (n=39) that was phenotypically evaluated for inducible clindamycin resistance. Subsequently, constitutive-inducible clindamycin (cMLSb) phenotypes was only detected in 8 (20.5%) *S. aureus* isolates. This was in accordance to the study conducted in Bangalore, India and in Port Harcourt, Nigeria [1,2] where some *S. aureus* clinical isolates were found to be inducibly resistant to clindamycin by the D-test technique. On average, the *S. aureus* isolates that were found to be inducibly resistant to clindamycin are resistant to 7 out of the 8 antibiotics used in this study (MARI of 0.7); and this shows the multiple antibiotic resistance nature of the isolates. This result of ours gives impetus to the possible emergence and spread of both cMLSb and iMLSb resistance *S. aureus* phenotypes in this part of the world. And the inability or non-detection of cMLSb and iMLSb phenotypes amongst *S. aureus* isolates as well as be on the lookout for other resistance phenotypes from clinical samples in hospital laboratories in Nigeria could result in treatment failure in our hospitals. It

is of utmost importance for Nigerian hospitals to be on the lookout for inducible-clindamycin resistance (iMLSb) and constitutive-clindamycin resistance (cMLSb) phenotypes amongst *S. aureus* isolates from clinical samples owing to the clinical importance of antibiotics in the MLSb family. The excellent pharmacokinetic and pharmacodynamics features of clindamycin makes it very attractive as the antibiotic of choice for treating infections caused by *S. aureus* [3,7,12]. However, the increasing frequency of therapeutic failures of clindamycin used for treating *S. aureus* infections especially those that were susceptible to it but actually resistant to erythromycin necessitates the need for clinical laboratories to always lookout for iMLSb and cMLSb in their routine work [1,5,8,9]. Our results show that some *S. aureus* clinical isolates are inducibly resistant to clindamycin which is an important antibiotic used clinically for the treatment of *S. aureus* infections. These *S. aureus* isolates were also found to be multiply resistant in nature. We therefore recommend the introduction of the D-test (for inducible clindamycin resistance detection) in our hospital's laboratory routine practice to detect inducible clindamycin resistance in clinical isolates of *S. aureus* – since routine antibiotic susceptibility tests cannot identify both cMLSb and iMLSb resistance strains.

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