

Inducible-Clindamycin Resistance in *Staphylococcus aureus* Isolates in Rivers State, Nigeria

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Abstract: Clindamycin is indicated in the treatment of skin and soft-tissue infections caused by Staphylococcal species. Treatment of an infection caused by a strain carrying inducible *erm* gene using clindamycin or any non-inducer macrolide can lead to clinical failure. The present study was aimed to detect inducible-clindamycin resistance (MLS_B_i) among *S. aureus* isolates in Port Harcourt, Nigeria and to study the relationship between clindamycin and methicillin-resistant *S. aureus* (MRSA). Two hundred and five (205) non-duplicate *Staphylococcus aureus* previously isolated from human sources were randomly collected from three health facilities- University of Port Harcourt Teaching Hospital, Braithwaite Memorial Specialist Hospital and De-Integrated Laboratories-all located in Port Harcourt, Nigeria, for this study from August, 2012 to July, 2013. Isolates were grouped as hospital in-patient (termed hospital-acquired – Nosocomial; n = 76) and out-patient cases (community-acquired; n = 129) *Staphylococcus aureus*. The isolates collected were reconfirmed following standard laboratory protocols. All confirmed isolates were stored in glycerol at +4°C (later sub-cultured for various phenotypic analyses). Using the disk diffusion method, detection of MRSA was carried out with 1µg of oxacillin (OXOID) placed on Mueller-Hinton agar with 4% NaCl supplementation. Antimicrobial susceptibility testing was performed using Erythromycin (15µg) and Clindamycin (2µg) both obtained from OXOID, UK. All clindamycin-sensitive isolates that were also erythromycin-resistant were subjected to D-Test phenotype (Inducible-clindamycin resistance). Among the 205 *S. aureus* isolates studied, Forty-four (21.5%) showed resistance to erythromycin, while 38 of these erythromycin-resistant isolates were simultaneously sensitive to clindamycin. Overall, out of 205 isolates, inducible-clindamycin resistance was detected in 23 (11.2%) of the isolates. These 23 (inducible MLS_B_i phenotype) are among 38 erythromycin-resistant *S. aureus* that were simultaneously sensitive (phenotypically) to clindamycin. Ten (4.9%) of the total (205) study isolates expressed constitutive resistance to clindamycin. Oxacillin Resistance (MRSA) was detected in 25 (12.2%) of the 205 isolates. Among the 38 erythromycin-resistant *S. aureus*, four were MRSA while 3 (75%) of the 4 erythromycin-resistant MRSA expressed inducible resistance to clindamycin. 20 (58.8%) of 34 erythromycin-resistant MSSA expressed inducible resistance to clindamycin. MRSA phenotype was not significantly correlated (p=0.9430) to inducible-clindamycin resistance. Inducible clindamycin-resistance often leads to treatment failure. The clinical microbiology laboratories in Nigeria should consider routine testing and reporting of inducible clindamycin resistance in *S. aureus*. There is also the need for sustained surveillance of antimicrobial susceptibilities of *S. aureus* in this region.

Keywords: *Staphylococcus aureus*, MRSA, Erythromycin- Resistance, Inducible-Clindamycin Resistance

1. Introduction

The determination of antimicrobial susceptibility pattern of a clinical isolate is very important for the effective management of infected patients. This is more so in the light

of the emergence of multidrug-resistant microorganisms. *Staphylococcus aureus* has the notoriety of being one of the most common organisms causing both hospital and community-acquired infections in many regions of the world. The increasing prevalence of methicillin resistance among *Staphylococci* is an increasing problem [1-4]. In view of the

gradual depletion in the armamentarium of antimicrobial agents effective against *S. aureus*, there has been renewed interest in the use of the Macrolide (erythromycin, clarithromycin)-Lincosamide (clindamycin, lincomycin)-Streptogramin B (quinupristin-dalfopristin) (MLS_B) antibiotics to treat *S. aureus* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties [5-6]. MLS_B antibiotics although structurally related, exhibit similar mode of action. They inhibit bacterial protein synthesis by binding to 23S rRNA, which is a part of large ribosomal subunit.

Resistance to macrolides (e.g. erythromycin) can occur by two different mechanisms: the efflux mechanism encoded by the gene- macrolide streptogramin resistance (*msrA*) and ribosome alteration or modification mechanism due to erythromycin ribosome methylase (*erm* gene) [7-8].

Target modification alters a site in 23S rRNA common to the binding of MLS_B antibiotics. Modification of the ribosomal target confers cross-resistance to MLS_B antibiotics. This cross-resistance, called the MLS-B phenotype, results from enzymatic methylation of an adenine residue of the 23S component of the 50S ribosomal subunit that these 3 drug groups bind to [9].

Expression of resistance to MLS_B in staphylococci may be constitutive (MLS_{Bc}) or inducible (MLS_{Bi}). In constitutive resistance, r-RNA methylase is always produced (MLS_{Bc}); whereas in inducible, methylase is produced only in the presence of an inducing agent (MLS_{Bi}) [10]. *In vitro*, *S. aureus* isolates with constitutive resistance are resistant to both erythromycin and clindamycin whereas those with inducible resistance are resistant to erythromycin and appear sensitive to clindamycin (MLS_{Bi}) [11].

For MLS_{Bi} strains, erythromycin will induce production of the methylase, which allows clindamycin resistance to be expressed. Inducible clindamycin resistance can then be detected with a simple disk approximation test, commonly referred as the D test [12]. When 15 µg erythromycin (E) or 2 µg clindamycin (C) are placed 15 to 20 mm apart on an agar plate that has been inoculated with the clinical isolate, the lack of a zone of inhibition around the erythromycin disc indicates bacterial resistance to macrolides (e.g. perhaps due to expression of a P-glycoprotein efflux pump that affects macrolides). The large clear zone of inhibition around the clindamycin disc indicates sensitivity to clindamycin. For a positive D-test, diffusion of erythromycin from the disc towards the clindamycin disc does not kill bacteria due to *S. aureus* resistance to macrolides. However, the bacterial isolate contains a strain of *S. aureus* with an erythromycin-inducible methylase (MLS_{Bi}) that is encoded by a plasmid-borne gene (*erm*). When this methylase is induced it alters the binding site on the 23S subunit of the 50S ribosome that both erythromycin and clindamycin bind to, making both antibiotics ineffective (inducing resistance). As a result, as erythromycin diffuses outward towards clindamycin, resistance to clindamycin is induced prior to its diffusion from the neighboring disk. In contrast, growth-inhibiting concentrations of clindamycin reach the zone near

erythromycin before erythromycin can arrive to induce resistance (due to the shorter distance for diffusion), resulting in inhibited growth. The inhibition of bacterial growth produces a “D” shape surrounding the clindamycin disk, which is considered a “positive” D-test. [9].

The treatment of patients harboring MLS_{Bi} staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure [13]. In case of another mechanism of resistance mediated through *msrA* genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin-resistant and clindamycin-sensitive both *in vivo* and *in vitro* and the strain do not typically become clindamycin resistant during therapy [6].

The present study was aimed to detect inducible clindamycin resistance (MLS_{Bi}) among *S. aureus* isolates in our geographical setting and to study the relationship between clindamycin and methicillin-resistant *S. aureus* (MRSA).

2. Materials and Methods

2.1. Study Area and Collection of Specimens

Two hundred and five (205) clinical isolates of *Staphylococcus aureus*, were collected between August, 2012 and July, 2013, from three health facilities- University of Port Harcourt Teaching Hospital, Braithwaite Memorial Specialist Hospital and De-Integrated Laboratories-all located in Port Harcourt, Rivers State of Nigeria, were used in this study. Isolates were previously cultivated from different specimens such as Urine, Blood, High Virginal Swab, Endo-cervical Swab, Intra- cervical Swab, Wound swab, Ear Swab, Eye Swab, Semen and other body fluids. Isolates were also grouped as Hospital in-patient or Out-patient isolates according to the criteria as prescribed by the Centers for Disease Control and Prevention [14]. Reconfirmation of isolates were done using colonial morphology on DNase agar plate, Mannitol salt agar plate, Gram stain, Catalase and Coagulase (bound / free) tests following standard protocols [15].

All confirmed isolates were stored at +4°C and later sub cultured to carry out antibiotic susceptibility testing against Oxacillin (1µg) (MRSA) and D-Test (Inducible clindamycin resistance) using Erythromycin (15µg) and Clindamycin (2µg).

2.2. Detection of MRSA

Disk diffusion tests as per the method described by Kirby and Bauer (1966) [16] were performed with 1µg of oxacillin (OXOID, UK) per disk placed on Mueller-Hinton agar (OXOID, UK) with 4% NaCl supplementation. The zone of inhibition was determined after 24 hours of incubation at 37°C. Organisms showing inhibition zones equal to or less than 10 mm were interpreted as resistant to oxacillin. Organisms with a zone equal to or greater than 12 mm were interpreted as susceptible while those with an inhibition zone of 11-12 mm were interpreted as intermediate. *S. aureus* strain ATCC 25923 was used as control.

2.3. Test for Macrolide Induction (D-Test)

Test was performed on all isolates of *Staphylococcus aureus* on Muller-Hinton agar (MH), using 2-hour-old nutrient broth culture. Briefly, inocula of bacteria were prepared to 0.5 McFarland standards. Sterile swab stick was dipped into the bacteria suspension and used to streak the MH agar. After seeding the agar plate with *S. aureus*, erythromycin (15µg) and clindamycin (2µg) disks, all obtained from Oxoid, UK, were placed 15-20 mm apart for the detection of inducible resistance of clindamycin by erythromycin (D-test) as previously described [12]. Plates were incubated at 37°C for 24 hrs. Inducible MLS_B phenotype (MLS_B_i) *S. aureus* isolates were interpreted as 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). Detection of a flattened zone correlates with presence of the *erm* gene and resistance to clindamycin even though it may appear susceptible to clindamycin by routine testing methods. *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 were interpreted as Constitutive MLS_B phenotype (MLS_B_C).

2.4. Data Analysis

All data were analyzed using the Chi square and t-tests. In addition, SPSS version 17.0 statistical package was employed. P-values of <0.05 were accepted as significant.

3. Results

Table 1. Prevalence of MRSA among *S. aureus* isolates in Port Harcourt.

Category	Number screened	MRSA (%)	MSSA (%)
Total no of <i>S. aureus</i> screened	205	25 (12.2)	180 (87.8)
Out- patient	129	7 (5.4)	122 (94.6)
In-patient	76	18 (23.7)	58 (76.3)
p-value		0.000318	0.179371

Two hundred and five (205) non-duplicate isolates of *Staphylococcus aureus* cultivated from different clinical specimens between August, 2012 and July, 2013,

were used in this study. The oxacillin disc susceptibility testing showed that 25 (12.2%) out of 205 isolates of *S. aureus* were resistant to oxacillin (Table 1).

Forty-four (36 MSSA and 8 MRSA) of the isolates showed resistance to erythromycin (Table 2).

Table 2. Antimicrobial Resistance Profile of *S. aureus* isolates Number (%) of Isolates resistant among.

Antimicrobial Agent	MSSA (n=180)	MRSA (n=25)	Total (n=205)
Erythromycin (15 µg)	36 (20)	8 (32)	44 (21.5)
Clindamycin (2 µg)	4 (2.2)	6 (24)	10 (4.9)

P<0.05

Table 3 shows the occurrence of constitutive clindamycin resistance among *S. aureus* isolates. Ten (4 MSSA and 6 MRSA) of the total (205) study isolates expressed constitutive resistance to clindamycin.

Table 3. Occurrence of Constitutive Clindamycin Resistance among *S. aureus*.

Population screened	No. screened	No. positive (%)	No. negative (%)	P – value
<i>S. aureus</i>	205	10 (4.9)	195 (95.1)	
MRSA	25	6 (24.0)	19 (76.0)	<0.05
MSSA	180	4 (4.0)	176 (96.0)	
In-patient	76	6 (7.9)	70 (92.1)	<0.05
Out-patient	129	4 (3.1)	125 (96.9)	

Table 4 shows inducible clindamycin resistance. Overall, 23 (11.2%) of the isolates, expressed inducible resistance. Furthermore, out of 38 erythromycin-resistant *S. aureus* that were simultaneously sensitive (phenotypically) to clindamycin in this study, 23 (60.5%) expressed inducible resistance to clindamycin (inducible MLS_B phenotype) (Table 4; Figure 1). There was no significant difference in MLS_B detection rate between Erythromycin-resistant MRSA and Erythromycin-resistant MSSA (p = 0.9430). Similarly, there was no significant difference in MLS_B detection rate between the corresponding in-patient and out-patient isolates (p = 0.3532).

Table 4. Occurrence of Inducible Clindamycin Resistance among *S. aureus* isolates.

	No screened	No. of positive (%)	No. of Negative (%)	P – value
<i>S. aureus</i>	205	23 (11.2)	182 (88.8)	
ERSA/CSSA				
Total population	38	23 (60.5)	15 (39.5)	
MRSA	4	3 (75.0)	1 (25.0)	0.942952976
MSSA	34	20 (58.8)	14 (41.2)	
In-patient	17	13 (76.5)	4 (23.5)	0.353237822
Out-patient	21	10 (47.6)	11 (52.4)	

ERSA = Erythromycin Resistant *S. aureus*

CSSA = Clindamycin Sensitive *S. aureus*

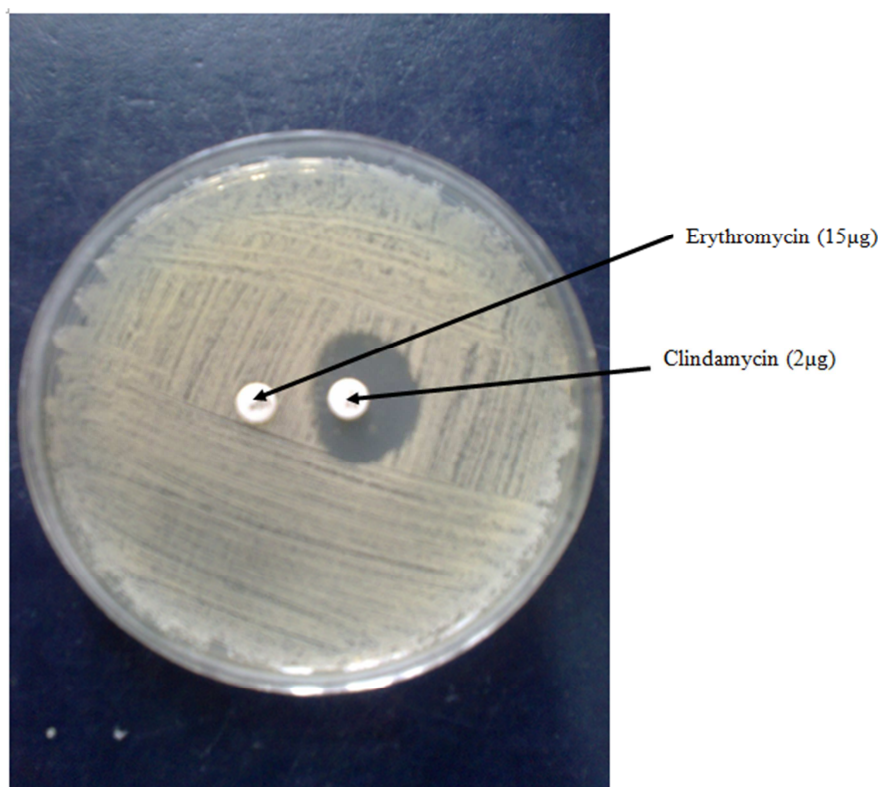


Figure 1. Inducible Clindamycin Resistance in *S. aureus*.

4. Discussion

Clindamycin is indicated in the treatment of skin and soft-tissue infections, caused by staphylococcal species. It is also used as an alternative for patients who are allergic to penicillin [11]. Treatment of an infection caused by a strain carrying inducible *erm* gene using clindamycin or any non-inducer macrolide can lead to clinical failure [11]. *In vitro* routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to *erm* genes resulting in treatment failure, thus necessitating the need to detect such resistance by a simple D test on a routine basis.

Among the 205 *S. aureus* isolates studied, Forty-four (21.5%) showed resistance to erythromycin (Table 2). This is higher than those reported by Ajantha *et al.*, 2008 [17] (15.7%) but lower than 28.4% [18] and 32.4% [6]. Erythromycin is one of the commonest and affordable antimicrobial agents available locally in Nigeria. An erythromycin- resistance rate of 21.5% in this study is actually a cause for concern. Out of 38 erythromycin-resistant *S. aureus* that were simultaneously sensitive (phenotypically) to clindamycin in this study, 23 (60.5%) expressed inducible resistance to clindamycin (inducible *MLS_B* phenotype) (Table 4). Overall, out of 205 isolates, inducible clindamycin resistance was observed in 23 (11.2%) of the isolates (Table 4). When compared to this study, higher rates of inducible clindamycin resistance had been reported: 50.6% [19] and 49% [17] as well as lower rate - 10.5% [18].

Ten (4 MSSA and 6 MRSA) of the total (205) study isolates expressed constitutive resistance to clindamycin

(Table 3). Furthermore, 3 (75%) of the four erythromycin-resistant MRSA expressed inducible resistance to clindamycin, while 20 (58.8%) of 34 erythromycin-resistant MSSA also expressed inducible resistance to clindamycin (Table 4).

Shittu *et al.*, (2012) [2] had reported 2 of 5 erythromycin-resistant MRSA as expressing inducible resistance to clindamycin in a study while in another related study in KwaZulu-Natal province, South Africa, all the 50 erythromycin-resistant MRSA were positive for inducible *MLS_B* resistance using the D-test method [20]

A survey in Pennsylvania, USA, observed that 68% of MSSA and 12.3% of MRSA were D-test positive [21]. Observations indicate that the incidence of constitutive and inducible *MLS_B* resistance in staphylococcal isolates varies by geographic region [1].

It becomes more worrisome when 23 out of 38 erythromycin-resistant *S. aureus*, as indicated in this study, are inducibly resistant to clindamycin. There appears to be paucity of literatures on *S. aureus* resistance to clindamycin in this locality probably due to oversight or complete ignorance. The D-test is a simple and reliable method to detect inducible resistance to clindamycin and has been so recommended by the National Committee for Clinical Laboratory Standards [22] as well as other investigators [23-24]. Reporting *S. aureus* as susceptible to clindamycin without verifying the inducible-clindamycin resistance status may result in institution of inappropriate clindamycin therapy and the consequent treatment failure. The clinical microbiology laboratories in Nigeria should therefore

consider routine testing and reporting of inducible clindamycin resistance in *S. aureus*. The proportion of MRSA with the inducible MLS_B phenotype (3 of 4 tested) (Table 4) indicates that clindamycin may not be a therapeutic option for the treatment of an infection attributed to an inducibly resistant MRSA. If clindamycin is used for treatment of infections with MLS_B-positive isolates, close follow-up and monitoring of failure or relapse is needed. However, in more severe infections, the presence of the MLS_B phenotype should preclude the use of clindamycin.

5. Conclusion

Inducible clindamycin-resistance often leads to treatment failure and therefore the detection rate in this study is instructive for clinical microbiology laboratories in Nigeria to consider routine testing and reporting of inducible clindamycin resistance in *S. aureus*. There is also the need for sustained surveillance of antimicrobial susceptibilities of *S. aureus* in this region.

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