Determination of Inducible Clindamycin Resistance Amongst Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* in Kaduna, Nigeria.

ABSTRACT

For quite a long time, mainly due to fewer treatment options, Clindamycin have been regarded as an alternative drug to effectively manage all *Staphylococcus aureus* infections. Strains with inducible clindamycin resistance (iMLS$_B$ phenotype) raise concerns because therapeutic failure may occur during treatment. Clindamycin is mostly regarded in treating serious infections that cannot be treated by other antibiotics, as it may cause colitis or mild diarrhea. A double disc diffusion test (D-test) for detecting inducible resistance to clindamycin in erythromycin-resistant methicillin-resistant *S. aureus* was performed by placing a 15µg erythromycin disc in proximity to a 2µg clindamycin disc in adjacent positions. For erythromycin-resistant isolates, D-test can help to determine whether clindamycin could be used as a therapeutic option (reported as susceptible when the D-test is negative or reported as resistant when the D-test is positive). Twenty (20) out of the 30 (thirty) isolates collected were confirmed to be determined by D-test as per CLSI guidelines. Fifteen (15) isolates were D-test Negative, 5 were resistant to both Erythromycin and Clindamycin, and none was D-test positive. D-test should be included as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance in staphylococci for the optimum treatment of patients.

Keywords: D-test, Erythromycin-resistance, iMLS$_B$ phenotype, Methicillin-resistance and Strains.

INTRODUCTION

There has been a limited choice for antibiotics used to treat infections associated with MRSA. Clindamycin, a lincosamide antibiotic is regarded as an alternative, mainly when other antibiotics fails in the treatment of MRSA associated infections (Back *et al.,* 2012). In the presence of erythromycin resistance there is the concern by clinicians on the use of clindamycin as it has been linked with the possible induction of cross resistance among members of streptogramin B (MLS$_B$) group, lincosamide and other macrolides (Capraro *et al.,* 2013). Clindamycin works primarily by binding to the 50s ribosomal subunit of bacteria. This agent disrupts protein synthesis by interfering with the transpeptidation reaction, which thereby inhibits early chain elongation (Goyal *et al.,* 2014; Back *et al.,* 2012).

Staphylococcus aureus is a major human pathogen and a global public health threat. It is considered an opportunistic pathogen as it asymptomatically colonizes its host, but can occasionally cause diseases that range in severity from relatively minor skin and soft tissue infections (SSTI) to life threatening cases of pneumonia and endocarditis (Schreckenberger *et al*., 2014). *S. aureus* is increasingly recognized as a cause of hospital associated (HA) and community associated (CA) infections. Macrolide, lincosamide and streptogramin B (MLSB)

antibiotics are commonly used in treatment of staphylococcal infections (Yilmaz and Aydin, 2007). Widespread use of MLSB antibiotics has led to an increase in resistance to these antibiotics especially clindamycin, amongst staphylococcal strains (Lim *et al*., 2012; Drinkovic *et al*., 2011; Lina *et al*., 2009). Macrolides such as erythromycin, roxithromycin, clarithromycin and lincosamides such as clindamycin and lincomycin belong to different classes of antimicrobials but act through the same mechanism that is by inhibition of protein synthesis (Steward *et al*., 2015). Clindamycin has long been an option for treating both methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) infections (Gadepalli *et al*., 2016).

The mechanism of clindamycin resistance may be constitutive, where methylase is always produced, or can be inducible, where methylase is produced only in presence of a macrolide inducer. Inducible clindamycin resistance is linked with drastic reduction in its effectiveness (Patel *et al*., 2016). Among macrolide, lincosamide and streptogramin B drugs only macrolides are good inducers of the enzyme erythromycin ribosome methylase (*erm*). Once induced, the gene product confers cross-resistance to other members of the group including lincosamides and streptogramin B (Leclercq, 2012). *S. aureus* isolates with constitutive resistance show resistance to erythromycin and clindamycin on in vitro testing, whereas isolates with inducible resistance show resistance to erythromycin but appear sensitive to clindamycin on disc diffusion testing (Goyal *et al.*, 2014; Sanchez, 2013).

METHODOLOGY

Sample Collection

Thirty (30) isolates of suspected methicillin-resistant *S. aureus* were collected from National Ear Care Centre (NECC), Kaduna. Immediately after collection, the isolates were transported aseptically to the Microbiology Laboratory, Department of Microbiology, Kaduna State University.

Media preparation

The most commonly used media that support the growth of *Staphylococcus aureus*, Mannitol Salt Agar was prepared according to manufacturer's instruction.

Isolation of pure cultures

The Method as described by Cheesbrough (2017) was adopted. Prepared Nutrient agar was poured in plates, allowed to solidify followed by the inoculation of the confirmed isolate and incubated at 37^{o}C for 18-24hrs. From the incubated plates, colony morphology of different colonies were carefully observed. Single-well isolated colony was then transferred aseptically on to the surface of a prepared nutrient agar plates and incubated at 37^{o}C for 24hrs. Colonies which were present only on the streak lines were noted and were further sub-cultured on to the surface

of the prepared agar slant in the bijou bottles for storage. The stored pure culture were used for characterization and identification.

Re-confirmation of *S. aureus*

The isolates were confirmed by conventional methods (colony morphology, Gram stain, coagulase, Oxidase and catalase tests, growth on selective medium and fermentation of mannitol) (**Cheesbrough**, 2017).

Determination of Methicillin Resistance in *Staphylococcus aureus*

Disk diffusion tests as per the method described by Kirby and Bauer (Clinical and Laboratory Standard Institute, 2016) was performed with 1µg of oxacillin per disk, placed on Mueller-Hinton agar with 4% NaCl supplementation. The zone of inhibition was determined after 24hours of incubation at 37°C. Organisms showing inhibition zones equal to or less than 10 mm were interpreted as resistant to oxacillin.

Erythromycin Susceptibility Testing

The Methicillin-resistant (MRSA) isolates were subjected to susceptibility test using Kirby Bauer disc diffusion method on Mueller Hinton agar plates using Erythromycin (15µg). The result was interpreted as per clinical laboratory standard Institute (CLSI) guidelines (CLSI, 2016).

Determination of Inducible Clindamycin Resistance

Isolates that were erythromycin resistant, were tested for inducible resistance by the D-test as per CLSI guidelines (CLSI, 2016). Erythromycin (15µg) disc was placed at a distance of 15mm (edge) from clindamycin (2µg) on Mueller Hinton agar plates previously inoculated with 0.5 McFarland bacterial suspension. Isolates that were erythromycin resistant and clindamycin susceptible with a D-shape inhibition zone around the clindamycin disc, were considered to be positive (iMLS$_B$ phenotype) but isolate that were erythromycin resistant and clindamycin susceptible with both zones of inhibition showing a circular shape, were considered as negative for inducible resistance (D-test negative, MS phenotypes).

RESULTS AND DISCUSSION

In this study, out of the 30 isolates of *S. aureus* collected, 66.6% (20/30) had inhibition zones of less than or equal to 10mm and as such were confirmed to be methicillin-resistant *S. aureus* (MRSA), while 33.3% (10/20) had inhibition zones of greater than or equal to 12mm, hence, were Methicillin susceptible *S. aureus* (MSSA) (Table 1). The confirmed Methicillin-resistant *Staphylococcus aureus* obtained were also resistant to erythromycin by showing zone diameter less than or equal to 13mm (Table 2). All the isolates were erythromycin resistant, 15 isolates were D-test negative while 5 were constitutive MSL$_B$ phenotype, which may be due to the fact

that, they do not harbor an *er3m* gene responsible for inducing resistance to macrolides. All the 66.6% (20/30) tested MRSA isolates studied showed resistance to erythromycin.

All the samples that were methicillin and erythromycin resistant were further tested for inducible clindamycin resistance. None of the 20 sample was D-test positive, while 15 were D-test negative and 5 were resistant phenotype (Constitutive MLS_B- grow up to clindamycin and erythromycin) (Table 3). Out of 20 erythromycin-resistant *S. aureus* that were simultaneously sensitive (phenotypically) to clindamycin in this study, none expressed inducible resistance to clindamycin (inducible MLS_B phenotype). This is in disagreement with the work of Shittu and Lin (2006) and Drinkovic *et al.* (2011) who reported 2 of 5 erythromycin-resistant MRSA as expressin inducible resistance to clindamycin in a study wile in another related study in KwaZulu-Natal province, South Afriuca, all the 50 erythromycin-resistant MRSA were positive for inducible MLS_B resistance using the D-Test method (Fiebelkorn *et al.*, 2013; Shittu and Lin, 2006).

Erythromycin is one of the commonest and affordable antimicrobial agents available locally in Nigeria. An erythromycin-resistance rate of 66.6% in this Study is actually a cause for concern. Use of D-test in a routine laboratory will help in guiding the clinicians regarding the judicious use of clindamycin in skin and soft tissue infections; as clindamycin is not suitable drug for D-test isolates but can be a drug of choice in the case of D-test negative isolates (Drummond *et al.*, 2003).

Staphylococcal strains resistant to macrolides and type-B streptogramins (MS phenotype) frequently harbor *msrA* gene, which encodes an ATP-dependent efflux pump (Hamilton-Miller and Shah, 2010). Isolates with *msrA*-mediated efflux also appear erythromycin resistant and clindamycin susceptible *in vitro* just like with those harboring *erm* gene, however they do not become erythromycin resistant during therapy. This study findings are different with studies published by other authors. Gunduz *et al.* showed 6.5% inducible clindamycin resistance in Methicillin-resistant *Staphylococcal* isolates. Saderi *et al*. from Iran showed 6.4% inducible clindamycin resistance in the isolates of MRSA (Saderi *et al.*, 2011), while another study from Iran also reported 9.7% inducible clindamycin resistance among the isolates of MRSA (Rahbar and Hajia, 2002). Fasih *et al.* reported high percentage (72%) of inducible clindamycin resistance phenotype among the discordant (Clindamycin-sensitive, Erythromycin-resistant) isolate of MRSA (Fasih *et al.*, 2010). Fokas *et al.* from Greece documented 35% inducible clindamycin resistance among isolates of MRSA (Kaur and Sanjay, 2015). These result indicate that inducible clindamycin resistance phenotypr may vary in different hospital setups.

With respect to methicillin resistance in *S. aureus* isolates, this result 66.6% are also different from other authors. Vivek *et al.*, Fasih *et al*., and Seifi *et al*. reported 32.5%, 36%, and 41.7% methicillin resistance in the isolates of *S. aureus* respectively (Fasih *et al.*, 2005; Nicolas *et al.*,

2008; Maree *et al.*, 2007). Moreover, Cetin *et al.* fron Turkey reported a very high percentage of the overall nonjudicious use of oxacillin in these setups. One of the therapeutic options in the treatment of MRSA is considered to be clindamycin (Martines-Aguillar *et al.*, 2003). It was noticed that clindamycin cannot be considered as useful treatment option for hospital-associated MRSA infections. Clindamycin can be considered as an effective treatment option inD-test negative MRSA isolates especially in lower respiratory tract isolates in the situation of aspiration pneumonia where anaerobic cover is also required. Clindamycin also has good anaerobic activity (Miller *et al.*, 2015; Martinez-Aguillar *et al.*, 2003).

Table 1: Methicillin Susceptibility Pattern of Clinical Isolates

Zone of Inhibition (mm)	**Number of Isolates (N=30)**
R= ≤ 10mm	20(66.7%)
I= 11-12mm	**0**
S= ≥ 12mm	10(33.3%)

Key: R= Resistance, S= Susceptible, I= Intermediate, N= Total Number of samples

Table 2: Erythromycin Susceptibility Pattern of MRSA

Zone of Inhibition (mm)	**Number of Isolates (%)(N=20)**
R= ≤ 13mm	20(66.7%)
I= 14-17mm	0
S= ≥ 18mm	0

Key: R= Resistant, S = Susceptible, I= Intermediate, N = Total Number of samples

Table 3: MLS_B Resistance Phenotype of MRSA

Phenotype	**Number of Isolates (%)(N=20)**

Constitutive MLS_B Resistance (CLI-R, ERY-R)	5(25%)
Inducible MLS_B Resistance (CLI- R, ERY- S D+ve)	0
MS phenotype ERY- R, CLI- S, D-ve)	15(75%)

Key: ERY= Erythromycin, CLI= Clindamycin, R= Resistant, S= Susceptible, -ve = Negative, +ve = Positive, MLS_B = Macrolides, Lincosamides Streptogramin B

CONCLUSION

A total of 66.6% (20/30) were methicillin-resistant *S. aureus* while 33.3% (10/20) were Methicillin susceptible *S. aureus* (MSSA). The Methicillin-resistant *S. aureus* obtained were also resistant to erythromycin. None of the 20 sample was D-test positive, while 15 were negative and 5 were resistant phenotype (constitutive MLS_B).

RECOMMENDATION

i. Clinical microbiology laboratories should implement testing simple and effective D-test on all *Staphylococcus* species.
ii. D-test positive isolates should be reported clindamycin resistant to decrease treatment failure and clindamycin should not be used in treatment of the isolates tested D-test positive.
iii. Clindamycin should be used for management of *Staphylococcus aureus* with MS phenotypes.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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