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Detection of Inducible Clindamycin Resistance in Methicillin-Resistant *Staphylococcus aureus* Isolates from National Orthopaedic Hospital, Dala, Kano.

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Abstract
The effectiveness of the antibiotic (clindamycin) usually used in the treatment of skin, soft-tissue, bone and joints infections particularly those caused by Staphylococcal species is now put to test by the emergence of several methicillin-resistant *Staphylococcus aureus* (MRSA)strains carrying inducible *erm* gene.
This study intend to detect inducible-clindamycin resistance ($iMLS_B$) and MRSA among *Staphylococcus aureus* (*S. aureus*) isolates from orthopaedic patients.
Staphylococcus aureus strains were isolated from the nasal, wound, and urine specimen of the patients using standard microbiological techniques. Antibiotic susceptibility was carried out using the disc diffusion method for erythromycin, clindamycin and cefoxitin. Strains that are resistant to erythromycin and susceptible to clindamycin were selected to determine $iMLS_B$. The $iMLS_B$ was determined using the D-test phenotype by placing erythromycin and clindamycin 12-20 mm apart in order to induce *in-vivo* clindamycin resistance while MRSA was determined by the strains resistance to cefoxitin.
The isolation rate of *S. aureus* from the samples (n=134) was 26.8%. A total of 29 (80.5%) expressed constitutive resistance to erythromycin, and 31 (86.1%) expressed constitutive resistance to clindamycin. Five (5) erythromycin-resistant isolates were simultaneously sensitive to clindamycin. Inducible-clindamycin resistance was detected in 2 (5.5%) of the isolates. Cefoxitin resistance (MRSA) was detected in 34 (94.4%) of the isolates, all the erythromycin-resistant *S. aureus* and inducible-clindamycin resistant isolates were MRSA.
The resistance pattern observed and *erm gene* harboured by the isolates is a major challenge to the effectiveness of MLSB group of antimicrobial agents. Therefore, resistance should be prevented in order to preserve clindamycin as an optional treatment for skin and soft tissue infections.

Key words: *Staphylococcus aureus*, Inducible Clindamycin Resistance, MRSA, Orthopaedic Patients.

Introduction

The determination of bacterial resistance to antimicrobial agents is very important in detecting both phenotypic and genotypic genes responsible and understanding its virulence factors (Hughes and Anderson, 2017). *S. aureus* is among the most common organisms causing both hospital and community-acquired infection in the world. More so, *S. aureus* has been included among microorganism with multi-drug resistant isolates (van Duin and Paterson, 2016).
Globally, resistance to methicillin is on the increase as more MRSA strains continue to emerge within the hospital and community environments (Ventola, 2015). As more *S. aureus* strains become resistant to antimicrobial agents that were initially effective, interests have been diverted to the use of Macrolide (Erythromycin)-lincosamide (Clindamycin)-Streptogramin(Quinupristin dalfopristin) [MLS_B] antibiotics to treat skin and soft tissue infections (Uzun *et al*., 2014).
Clindamycin has been regarded as the preferred drug of choice due to its excellent pharmacokinetic properties (Smieja, 1998). Even though the MLS_B antibiotics are structurally related and exhibit similar mode of action, the protein synthesis is inhibited when binding occur in the 23s rRNA which is part of the large ribosomal subunit (Siibak *et al*., 2009).
Resistance to macrolide (erythromycin) usually occur by two different mechanisms: the efflux encoded by the gene macrolide streptogramin resistance (*msrA*) and ribosome alteration or modification mechanism due to erythromycin ribosome methylase (*ermgene*) [Easter and Samuel, 2016].
Resistance to MLS_B may be expressed as either constitutive (MLS_{Bc}) or inducible (MLS_{Bi}). In constitutive, rRNA methylase is always produced while in inducible, methylase is produced only in the presence of an inducing agent (Thapa and Sapkota, 2016). An *in vitro* test for *S. aureus*

isolates with constitutive resistance are resistant to both erythromycin and clindamycin while isolates with inducible resistance are resistant erythromycin and appear sensitive to clindamycin (Prabhu *et al*., 2011).

Report have shown that inducible-clindamycin resistance can be detected using the simple D test, when erythromycin (15µg) and clindamycin (2µg) are placed 12-20mm apart on an agar plate that has been pre-inoculated with the isolate (EUCAST, 2016). For MLS_{Bi} strains, the inhibition of bacteria will produce a "D" shape surrounding the clindamycin disc. The erythromycin induce production of methylase which allows clindamycin resistance to be expressed (Juyal *et al*., 2013).

Clindamycin may still be used as a short-term therapy for patients harbouring the MLS_{Bi} strains in less serious skin and soft tissue infections. Constitutive therapy is likely to occur during long-term therapy (EUCAST, 2016).

The aim of this study was to detect inducible clindamycin resistance and investigate MRSA strains among identified *S. aureus* isolates from orthopaedic patients. This research will help in making selection for appropriate antibiotics to be used for nasal decolonization and therapy of *S. aureus* infections.

Materials and methods

Sample Collection and Identification

In six different wards, 134 clinical samples were collected from nasal swabs, wound and urine specimens of orthopaedic patients in National Orthopaedic Hospital Dala, Kano during a period of three months (from September to December, 2016), *S. aureus* strains were identified on the basis of standard and conventional microbiological techniques which include gram staining, catalase test, coagulase test (Staphytect Plus Test Kit), then *Staphylococcus aureus* microgen identification kit (Microgen™ Staph-ID System).

Determination of Antibiotic Susceptibility

The disc diffusion method was used for erythromycin, clindamycin and cefoxitin to determine the antibiotic susceptibility of the *S. aureus* strains. Discrete colonies of isolates on nutrient agar plates were emulsified in 5 ml of sterile physiological saline and the turbidity adjusted to 0.5 McFarland standard (approximately a cell density of 1.5×10^8 cfu/ml) (Samie and Shivambu, 2011). The bacteria suspensions were cultured on a freshly prepared Muller Hinton agar plate, then antibiotic discs were applied, the plates were incubated invertedly at 37ºC for 18 hours. The plates were examined for the zones of inhibition and result interpretation according to EUCAST (2016).

Detection of Inducible Clindamycin Resistance

Isolates resistant to erythromycin and simultaneously susceptible to clindamycin were selected for this test, based on the initial antibiotic susceptibility test.

The inducible clindamycin resistance detection was carried out by D-zone test according to EUCAST (2016) guidelines. Erythromycin (15µg) disc was placed at a distance of 12 – 20mm edge to edge from clindamycin (2µg) disc on a Mueller Hinton agar plate, previously inoculated with 0.5 McFarland standard equivalent bacterial suspensions. After culturing at 37°C for 18 hours, flattening of the zone of inhibition adjacent to the erythromycin disc (referred to as a D-zone) known as *erm*-mediated inducible clindamycin resistance (positive D-test). While the absence of D-shaped and clindamycin growth inhibition zone diameter ≤14 is indicative of constitutive clindamycin resistance (Deresinski, 2005; Yilmaz *et al.*, 2007).

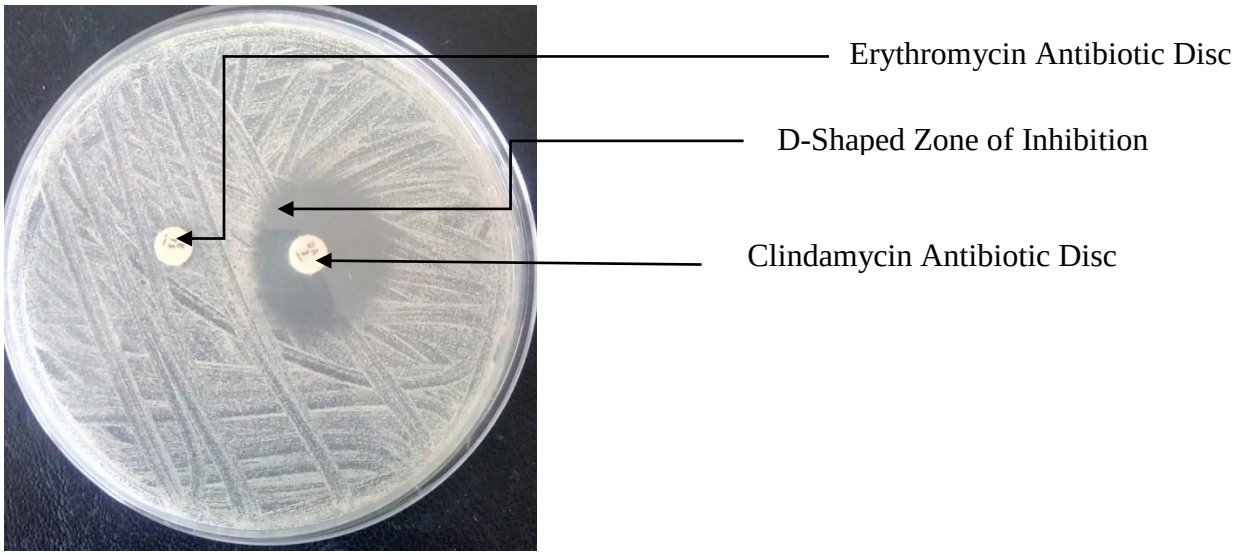

Figure 1: Inducible Clindamycin Resistance of *S. aureus* Isolates.

Results

The isolation rate of *S. aureus* from the samples (n=134) was 26.8%, mainly from nasal swab (36%) and wound swab (36%).

Majority of the *S. aureus* strains 34(94.4%) were detected to be MRSA and only 2(5.5%) were methicillin sensitive *S. aureus* (MSSA) as shown in table 1 below.

Table 1: Prevalence of *Staphylococcus aureus* Methicillin Susceptibility by Sample Type

Category	Number Screened	MRSA (%)	MSSA (%)
Nasal swabs	13	12 (33.3)	1 (2.7)
Wound swabs	13	13 (36)	0 (0)
Urine specimens	10	9 (25)	1 (2.7)
S. aureus	36	34 (94.4)	2(5.5)

Table 2: Antimicrobial Resistance Profile of MRSA Isolates against MLS_B Agents

Antimicrobial Agents	MRSA (n=34)	MSSA (n=2)
Erythromycin (15µg)	29 (85.2%)	2 (100%)
Clindamycin (2µg)	29 (85.2%)	0 (0%)
Quinupristin-dalfopristin (15µg)	22 (64.7%)	2 (100%)

All the MSSA strains were also sensitive to erythromycin and quinupristin-dalfopristin antibiotics, while all the MRSA were constitutively resistant to clindamycin as shown in table 2 above.

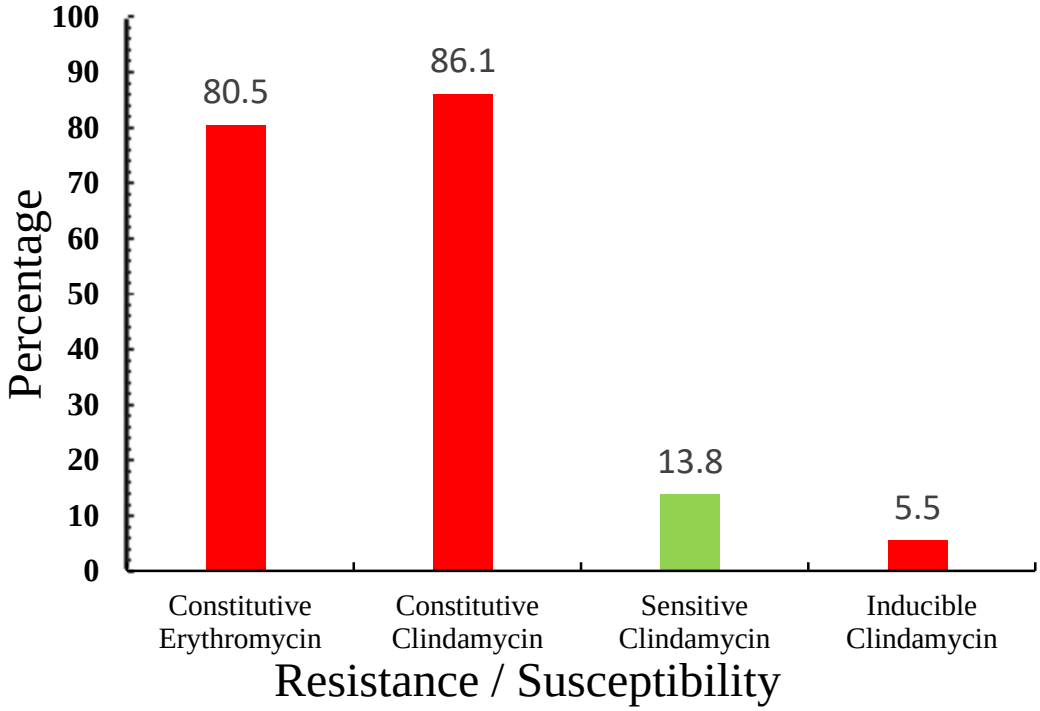

Figure 2: Prevalence of *S. aureus* Constitutive and Inducible-clindamycin Resistance

A total of 29 (80.5%) showed resistance to erythromycin, while 5 of these erythromycin-resistant isolates were simultaneously sensitive to clindamycin. Only 2 (5.5%) of the isolates showed Inducible-clindamycin resistance.

Discussion

In Kano, Adamu *et al*., (2009) reported similar isolation rate of 27% for *S. aureus* in clinical isolates. This showed that the prevalence of *S. aureus* from most hospitals in Kano have remained unchanged for a decade now.

Clindamycin is considered and advocated in severe in-patient MRSA infections depending on the antimicrobial susceptibility results. However, by using clindamycin, use of vancomycin can be avoided (Prabhu *et al*., 2011). Although, treatment of infections caused by strains carrying the *erm* gene using clindamycin or any non-inducer macrolide can lead to clinical failure (Easter *et al*., 2011).

The detection of the D-shaped pattern of resistance in figure 1, signifies the presence of *erm* genes in the isolates encoding for either inducible or constitutive resistance to clindamycin.

Kumurya, (2015) in Kano recorded 18.1% iMLS$_B$ which is higher than the observation in this study because they recorded a lower constitutive resistance of 46.9% to erythromycin and clindamycin. This suggest that clindamycin may no longer be an excellent drug for some staphylococcal infections particularly skin and soft tissue infections or as an alternative to penicillin-allergic patients in NOHD.

All the *S. aureus* strains resistant to MLS$_B$ Antimicrobial agents were also MRSA which is similar to the report of Mahmoud *et al*., (2015) where they stated that, iMLS$_B$ are found to be significantly higher among MRSA (43.5%) than MSSA (20.9%) strains. This will limit or render clindamycin ineffective for use as a therapeutic agent especially on strains regarded as MRSA in National Orthopaedic Hospital Dala, Kano.

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