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INDUCIBLE MACROLIDE RESISTANCE PHENOTYPES AND GENOTYPES IN CLINICAL ISOLATES OF *Staphylococcus aureus* FROM NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL, NNEWI, SOUTH EAST, NIGERIA

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ABSTRACT

Background: The widespread use of macrolide, lincosamide, and streptogramins-B (MLS-B) to treat Staphylococcal infections has caused an increase in resistance to these types of antibiotics.
Aim: The aim of this study is to identify macrolide resistant phenotypes and detect *erm* genes associated with macrolide resistance in *Staphylococcus aureus*.
Methodology: A total of 304 Gram positive cocci isolated from different clinical samples received at the Medical Microbiology Laboratory of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi were used for this study. Oxoid Staphytect agglutination test kits were used to confirm 185 of these isolates as *Staphylococcus aureus*. Antibiotic sensitivity testing was done on the 185 verified *S. aureus* isolates while the D-test was used to check for macrolide resistance. Twenty (20) of the D-test positive isolates were tested for the presence of *erm* A, B, and C genes using a multiplex PCR method.
Results: Results showed that the occurrence of inducible MLS-B phenotype was 23 out of 185 (12.4%) while 46 out of 185 (24.9%) of the isolates displayed the constitutive MLS-B phenotype. Out of the 20 resistant isolates tested for the presence of resistance genes, 8 (40%) tested positive for *erm* C, while none possessed either *erm* A or *erm* B genes. All 8 of the *erm* C positive isolates were resistant to methicillin (MRSA). The iMLS-B phenotypes were more frequently observed in isolates that tested positive for *erm* C compared to the cMLS-β phenotype.

Conclusion: This study stresses on the need to be aware that iMLSB and cMLSB phenotypes exist among clinical isolates of *S. aureus* and that resistant genes are found in some of these isolates. Such isolates should be sought for during routine laboratory investigations in order to avoid possible treatment failure.
Keywords: *Staphylococcus aureus*; macrolides; resistance; erm genes

Introduction

Staphylococcus aureus is a major cause of infections acquired in healthcare settings and is a prevalent bacterium that causes many types of infections in people of all ages[1]. It is a very effective bacteria that often colonizes the skin and mucosa of people and animals especially in the nose, on the skin, or both areas, causing infections when given the opportunity. Outbreaks of infections can occur with community outbreaks often linked to inadequate hygiene and the spread of germs from person to person through objects. Hospital outbreaks caused by one type of *S. aureus* strain typically affects patients who have had surgery or other invasive treatments.

Macrolides, like erythromycin, azithromycin, and clarithromycin; lincosamides such as clindamycin, and streptogramin-β like quinupristin are classes of antibiotics called MLS-β. They affect the 23S rRNA of the big 50S ribosomal subunit, which stops protein production[2]. The MLS-β group of antibiotics is one of the few remaining options to treat skin and soft tissue infections caused by both methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA). The widespread use of macrolide, lincosamide, and streptogramins-β (MLS-β) antibiotics in treating staphylococcal infections has caused a rise in resistance to these antibiotic families. Staphylococci mostly develop resistance to these antibiotics by efflux pumps[3], target change and drug inactivation[4].

Methicillin resistant *S. aureus* are clinically important pathogens because of their ability to resist all beta-lactam and other antibacterial agents thereby limiting treatment options for MRSA infections[5]. As MRSA are associated with myriads of infections in healthcare facilities as well as in the community, knowledge of the local antibiotic resistance patterns and carriage of virulence genes by these strains can enhance better treatment outcomes, and the control and/or prevention of infections[6,7].

It has been observed that in Nigeria, testing for antibiotic susceptibility of isolates usually relies on phenotypic testing and is hindered by the absence of evaluation of the molecular mechanism behind the resistance to the drugs. In addition, recent research have used PCR to detect *erm* genes to identify and confirm macrolide resistance in *S. aureus*, however; information on the genetic factors of macrolide resistance in *S. aureus* in Nigeria is somewhat limited[7,8,9]. So, using molecular screening to find the genetic cause of *S. aureus* resistance to macrolides will be very useful in reducing and implementing adequate control measures against infections caused by *S. aureus* bacteria. This study therefore sought for macrolide resistance in *S. aureus* isolated from clinical samples from Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi and also focused on detecting the presence of the erm genes responsible for target site modification in the bacteria. The result is expected to aid clinicians decide on the right therapy choices for most infections caused by *S. aureus*.

Materials and Methods

Three hundred and four (304) Gram positive coccal-shaped bacteria isolated from different clinical samples received at the Medical Microbiology Laboratory of NAUTH were used for this study. All the isolates were presumptively re-identified using standard microbiology techniques, including colony morphology on growth media, Gram staining, catalase, coagulase and DNAse tests. Of these, 185 were confirmed to be *S. aureus* using the Oxoid Staphytect agglutination test kits[10].

The Kirby–Bauer disk diffusion method was used to evaluate the antimicrobial susceptibility profiles of the confirmed *S. aureus* isolates, using a standardized single antibiotic disk on Oxoid Mueller–Hinton agar (MHA) as described by[11.12.] Various commercially available antibiotic discs (Oxoid Ltd Basingstoke) were used to determine susceptibilities of the isolates to the test antibiotics[10]. The diameter of the zone of inhibition for each antibiotic produced by the *S. aureus* isolates was measured in millimetres (mm), and this was considered as either sensitive or resistant to the test antibiotics based on the documented breakpoint guidelines of the CLSI standard interpretive criteria[13].

Methicillin resistance was evaluated using 30µg of cefoxitin. Isolates that had an inhibition diameter of ≥ 22mm were reported as methicillin susceptible *S. aureus* (MSSA) while those with zone diameters of ≤ 21mm were reported as methicillin resistant *S. aureus* (MRSA). This was done by measuring the inhibition zone diameter around the cefoxitin disc.

Double disk diffusion test (D-test) was used to screen for macrolide resistant phenotypes[14]. Isolates that were resistant to erythromycin but were susceptible to clindamycin with flattening or blunting of the inhibition zone around the clindamycin disc in a D-shaped form (D-Test positive) were reported as inducible-MLS-β (iMLS-β) phenotype. Also, MS phenotype was reported for isolates that showed resistance to erythromycin with no flattening or blunting of the inhibition zone around the clindamycin disc in a D-shaped form (D-Test negative). D-test positive isolates were screened for the presence of *erm* A, *erm* B and *erm* C genes using multiplex Polymerase Chain Reaction (PCR) technique.

Data collected was computed and statistical analysis was carried out using Statistical Package for Social Sciences version 22.0 (SPSS Chicago, USA). Frequency distribution tables and figures were used to show results.

Results

Among the 185 confirmed *S. aureus* isolates, 167 (90.3%) were found to be resistant to erythromycin, 165 (89. %) were resistant to cefoxitin and the least number of resistant isolates were to linezolide 48 (25.9%) (Table 1). Forty-four isolates from the 167 erythromycin resistant isolates were susceptible to clindamycin (26.3%). The D-test conducted on these 44 erythromycin resistant, clindamycin susceptible isolates revealed that 23 (52.3%) isolates were positive for the D-Test (Table 2).

In all, inducible macrolide resistance (inducible MLS-B phenotype) was demonstrated by 23 out of 185 (12.4%) *S. aureus* isolates, while 46 out of 185 (24.9%) *S. aureus* isolates were resistant to both erythromycin, clindamycin and linezolide (constitutive MLS-B phenotype). Also, 2 (1.1%) isolates were resistant to erythromycin and linezolide (MS-B phenotype) while 8 (4.3%) isolates were sensitive to erythromycin but resistant to clindamycin (L-phenotype) (Table 3).

Among the 165 MRSA isolates, 46 (27.9%) had constitutive MLS-B (cMLS-B)

phenotype while 38 (23.0%) showed inducible MLS-B (iMLS-B) phenotype. In addition, 6 (30%) of the 20 MSSA isolates showed iMLS-B phenotype while the cMLS-B phenotype was absent in MSSA isolates (Table 3). The resistance profile of the 165 Methicillin resistant *Stapylococcus aureus* (MRSA) isolates are shown in Figure 1.

The findings from the molecular screening for macrolide resistance genes in Figure 2 indicate that out of the 20 D-test positive *S. aureus* isolates that were screened for macrolide resistance genes, 8 (40%) isolates were positive for *erm C* gene. Of the 20 isolates also, none had the *erm A* or *erm B* genes (Figure 2). Also, all of the 8 *erm C* positive isolates were resistant to cefoxitin (MRSA).

Table 1: Resistance/Susceptibility profile of *S. aureus* to different tested Antibiotic classes (N=185)

Antibiotic type	Antibiotic Class	No. Susceptible (%)	No. Resistance (%)
Erythromycin	Macrolide	18 (9.7)	167 (90.3)
Clindamycin	Lincosanimide	54 (29.2)	131 (70.8)
Linozolide	Streptogramin-B	137 (74.1)	48 (25.9)
Cefoxitin	Cephalosporin	20 (10.8)	165 (89.1)
Ciprofloxacin	Fluoroquinolone	49 (26.5)	136 (73.5)
Trimetoprim/ Sulpumethoxazole	Folate Antagonist	50 (27)	135 (73)
Quinupristin/ Dalfopristin	Oxazolidinone	136 (73.5)	49 (26.5)
Gentamycin	Aminoglycoside	82 (44.3)	103 (55.7)

Table 2: Antimicrobial Susceptibility Test Result of the D-Test Positive Isolates (N = 44)

Antibiotic Class	SUSCEPTIBLE No. (%)	RESISTANT No. (%)
Erythromycin	0 (0)	44 (100)
Clindamycin	44 (100)	0 (0)
Linezolide	42 (95.5)	2 (4.5)
D-Test	23 (52.3)	21 (47.7)

Table 3: Occurrence of macrolide phenotypes in *S. aureus* isolates

Type of Macrolide phenotype	No. (%) of total isolates (N=185)	No. (%) of MRSA isolates (N=165)	No. (%) of MSSA isolates (N=20)
iMLS-β Phenotype	23 (12.4)	38 (23.0)	6 (30)
cMLS-β Phenotype	46 (24.9)	46 (27.9)	0(0)
MS-β Phenotype	2 (1.1)	0(0)	0(0)
L- Phenotype	8(4.3)	0(0)	0(0)

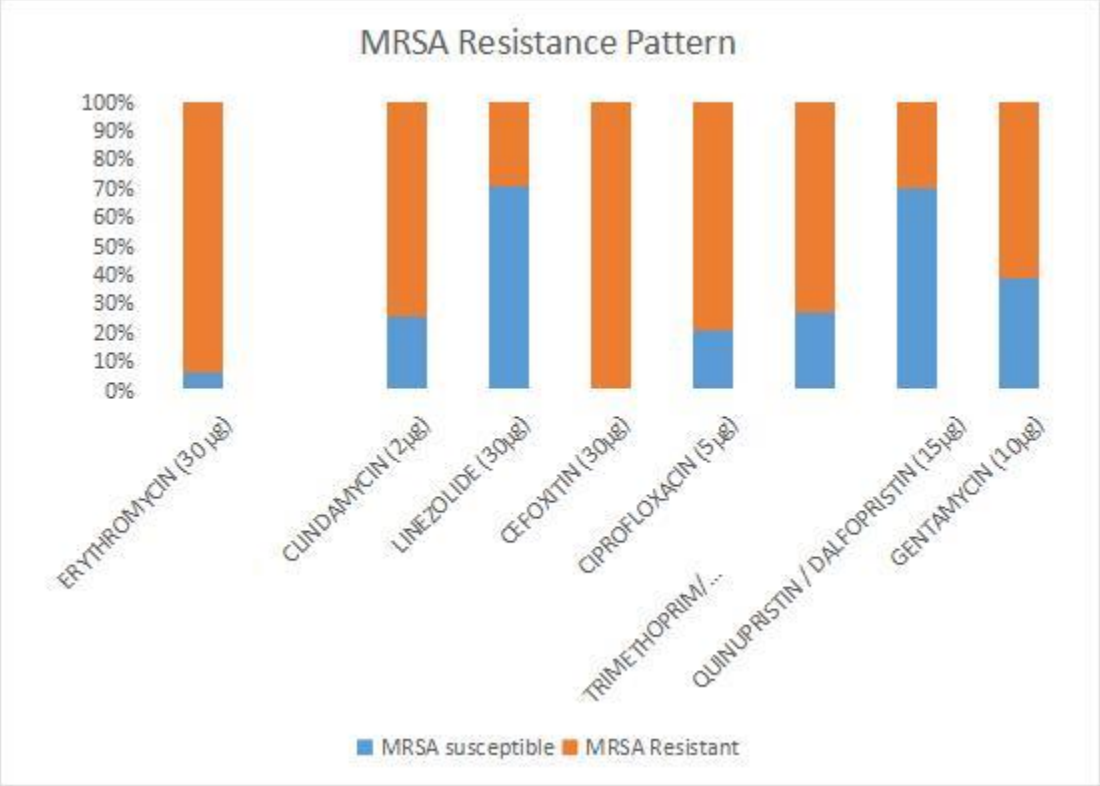

Fig 1: **Resistance Profile of MRSA**

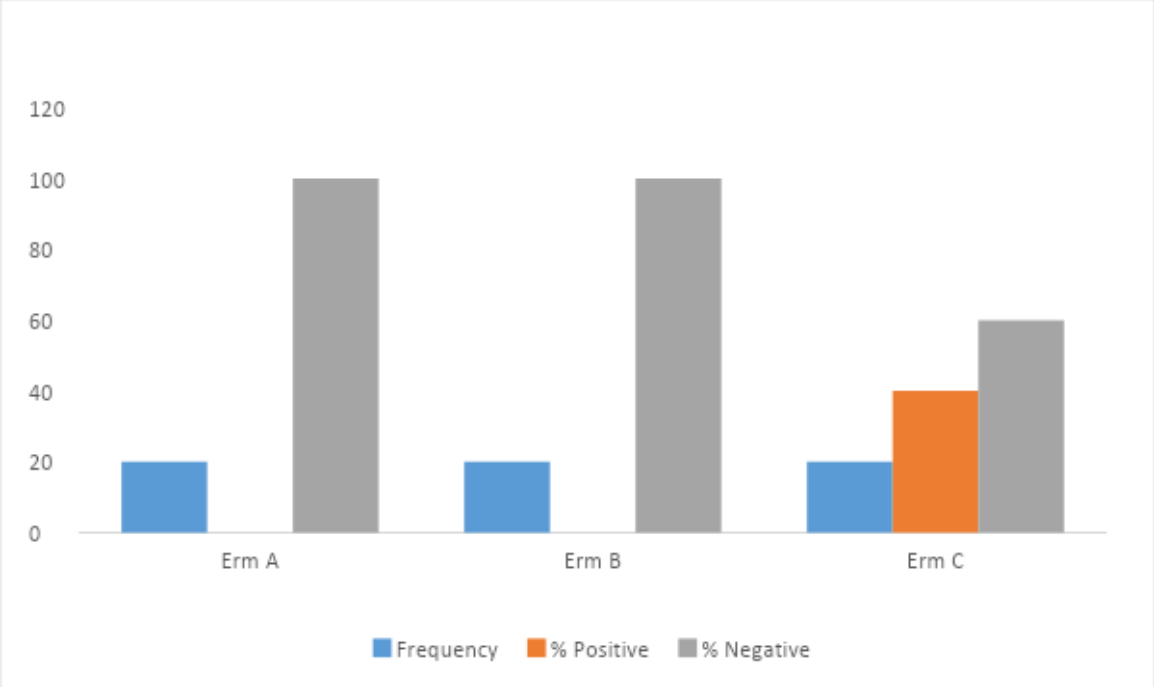

Figure 2: **Distribution of *erm* Gene in *S. aureus* Isolates**

Discussion

The results of this study shows that the overall prevalence of inducible (12.4%) and constitutive (24.9%) clindamycin resistance (iMLS-β and cMLS-β) among *S. aureus* isolates was consistent with the findings of another researcher who reported an overall prevalence of iMLS-β and cMLS-β resistance as 15.4 % and 20.5 % respectively[15]. Likewise, the high prevalence of erythromycin and clindamycin resistance obtained in this study with 90.3% and 70.8% respectively, is similar to those with overall prevalence of 74.4% and 76.9% for erythromycin and clindamycin respectively[15]. Two previous studies in Iran and Nepal recorded equally high inducible clindamycin resistance of 12.5% and 23.4% respectively[16,17]. These findings are contrary to those of other investigators who recorded an iMLS-β prevalence of 5.4 % and a cMLS-β prevalence of 6.2%[17]. These differences could be due to function of accuracy of diagnosis, study population[17], variation in geographical locations and specific characteristics of the healthcare facility.

Very high prevalences for erythromycin and cefoxitin resistance of 90.3% and 89.2% respectively were recorded in the present study, as was similarly reported by[9,18.]. Resistance to macrolides, lincosamides, and streptogramin-B (MLS-B) in most strains of *Staphylococcus aureus* correlates with resistance to methicillin[19]. This report corresponds with the findings of our study where 27.9% of the MRSA isolates exhibited cMLS-B resistance while 23.0% had iMLS-B resistance. These findings suggest that methicillin resistance in *S.*

aureus equally leads to resistance to other antibiotics, particularly macrolides. Macrolide-resistant methicillin resistant *Staphylococcus aureus* (MR-MRSA) strains pose a serious health issues because they are mostly implicated in difficult-to-treat infections that usually result in higher treatment cost and longer hospital stays[19].

The 40% prevalence recorded for *erm* C and non-availability of other *erm* genes indicates that *erm* C is the most dominant resistant gene in this part of the world. *Erm* C has been reported to be the most dominant macrolide resistance gene in Africa and Asia[20]. Other macrolide resistance genes, *erm* A and *erm* B, have been reported to be most dominant in Tunisia, Denmark and United Kingdom[16]. Most studies in Nigeria seek to determine the prevalence of inducible clindamycin resistance in clinical isolates of *S. aureus***,** hence there is limited data on the genetic factors of macrolide resistance in clinical isolates of *S. aureus* in this region.

The findings from this study show that iMLS-B phenotypes were more frequently observed among *erm* C positive isolates, compared to the cMLS-B phenotype. This could be due to the transfer of resistance genes between isolates through horizontal gene transfer, as macrolide resistance genes are usually found on mobile genetic elements[21]. Additionally, all the *erm* C positive isolates were resistant to cefoxitin, indicating that MRSA has a resistance to multiple drugs.

The high prevalence of MLS-B resistant genes found in isolates from this study may be due to the transfer of resistant genes among isolates. The non-detection of cMLS-B and iMLS-B phenotypes amongst *S. aureus* isolates as well as inability to look out 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 (iMLS-B) and constitutive-clindamycin resistance (cMLS-B) phenotypes amongst *S. aureus* isolates from clinical samples owing to the clinical importance of antibiotics in the MLS-B family.

Conclusion

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 include screening for iMLS-B and cMLS-B in their routine work. Therefore, the use of correct diagnostic methods and the right antibiotic for treating macrolide-resistant methicillin-resistant *S. aureus* (MR-MRSA) isolates will not only reduce mortality in patients but also preserve the few remaining alternatives for future use.

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