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INDUCIBLE CLINDAMYCIN RESISTANCE IN CLINICAL ISOLATES OF *Staphylococcus aureus* IN KATSINA

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ABSTRACT
Gram-positive organisms commonly exhibit resistance to macrolides, lincosamide, and streptogramin B (MLS_B), which is a prevailing and medically important mechanism. This resistance can manifest as constitutive ($cMLS_B$ phenotype) or inducible ($iMLS_B$ phenotype). Distinguishing between the two $iMLS_B$ phenotypes is challenging using routine susceptibility tests. However, the erythromycin-clindamycin disk approximation test (D-test) can effectively identify them. This study aimed to detect inducible clindamycin resistance in clinical Staphylococcus aureus isolates from Katsina. A total of 100 isolates were collected from three major hospitals in the Katsina metropolis and subsequently characterized using standard bacteriological techniques. Antibiotic susceptibility testing was performed following the modified Kirby Bauer disc diffusion method using erythromycin (15µg) and clindamycin (2µg). While cefoxitin (30µg) was used for phenotypic detection of MRSA. The D-test was used to evaluate the $iMLS_B$ phenotypes among isolates resistant to erythromycin and susceptible to clindamycin. Forty-nine isolates (49/100) were confirmed to be Staphylococcus aureus. Among the 49 S. aureus confirmed, fifteen (30.6%) were resistant to erythromycin, out of which 12 were concurrently susceptible to clindamycin. The prevalence of $iMLS_B$ phenotype was 25%, macrolide streptogramin (MS) phenotype 18.4%, and $cMLS_B$ 6.1%. Methicillin resistance (MRSA) was detected among 44.9% (22/49) of the isolates. All the detected $iMLS_B$ phenotypes were also MRSA. Consequently, the D-test was found to be crucial for identifying $iMLS_B$. It is recommended that D-test should be done routinely on S. aureus isolates that are clindamycin-susceptible and erythromycin-resistant.
Keywords: D-test, erm Genes, Inducible Clindamycin Resistance, Antibiotics and MLS_B

INTRODUCTION

Staphylococcal bacteria, which are commonly found on the skin and mucous membranes of healthy people, have the tendency to act as opportunistic pathogens. They typically cause infections in individuals who are more susceptible or predisposed to such infections (Michalik *et al.,* 2020). The majority of hospital-acquired infections, which can be severe and potentially fatal, are primarily caused by virulent strains of these bacteria, with methicillin-resistant *Staphylococcus aureus* (MRSA) being particularly noteworthy (Sikora and Zahra, 2021). MSRA is a major cause for concern due to its resistance not only to methicillin but also to a wide range of other chemotherapeutic agents (Velvizhi *et al.,* 2011). The treatment of MRSA infections now involves the use of antibiotics from the macrolides group (such as erythromycin, clarithromycin, and azithromycin), lincosamides (like clindamycin), and streptogramin B (quinupristin) - collectively known as the MLS_B group of antibiotics (Harkins *et al.,* 2017; Heyar *et al.,* 2020). Previous researches has documented that MLS_B resistance is facilitated by erythromycin ribosomal methylase genes (*erm*), which modify the shared drug binding sites of the three drug classes on the 23S rRNA (Moosavian *et al.,* 2014; Heyar *et al.,* 2020; Anandabaskar, 2021). MLS_B resistance can manifest in two forms: inducible, characterized by resistance to erythromycin while remaining susceptible to clindamycin, and constitutive, where resistance is observed against all MLS_B antibiotics (Leclercq, 2002; Moosavian *et al.,* 2014). Differentiating between the two MLS_B phenotypes can be difficult through routine susceptibility tests alone. However, the erythromycin-clindamycin double disk approximation test (D-test) is a reliable method for effectively identifying and distinguishing between the two phenotypes (CLSI, 2022). The objective of this study was to identify and assess the presence of inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus* from Katsina. The study was conducted due to a lack of available data on the prevalence of $iMLS_B$ (inducible macrolide-lincosamides-streptogramin B) resistance among *Staphylococcus aureus* isolates in this particular region.

MATERIALS AND METHODS

During the six months' study, a total of 100 non-repetitive clinical isolates of *Staphylococcus aureus* were collected from three hospitals located within Katsina metropolis; General Hospital Katsina (GHK), General Amadi Rimi Specialist Hospital (GARSH) and Turai Umar Musa Yar'adua Women and Children Specialist Hospital (TWCSH) after receipt of full ethical clearance certificate from the health research ethical

review committee of Katsina State ministry of health under HREC number MOH/ADM/SUB/1152/1/530. GHK is the apex State Government-owned healthcare centre while TWCSH and GARSH are specialist medical centres that serve mainly women and children healthcare needs and orthopaedic cases respectively. The isolates were previously isolated from wound swabs, urine, urogenital swabs, aspirates, blood and sputum. Subsequently, all the isolates were re-characterized using standard bacteriological techniques including colonial morphology on growth media (mannitol salt agar and blood agar), coagulase test, catalase test and Gram staining technique (Cheesbrough, 2009, Bannerman, 2003). *Staphylococcus aureus* ATCC33862 was used as a reference control organism and for result validation. All confirmed *Staphylococcus aureus* isolates were stored in 20% v/v glycerol broth at -20^{o}C for subsequent analysis. Throughout the study, the modified Kirby Bauer disk diffusion method, in accordance with the guidelines set by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2022), was employed. This method was utilized for various procedures including inoculum preparation, inoculation of test plates, and the application of antibiotic disks on Mueller-Hinton Agar (MHA). The use of CLSI guidelines ensured standardized and consistent testing protocols for accurate and reliable results.

Detection of MRSA and MSSA

The surrogate cefoxitin disc diffusion method was used for detection of *mec*A-mediated methicillin resistance. Cefoxitin (30µg) (Oxoid, UK) disks were placed on the surface of the agar. Plates were then incubated in an inverted position at 35^{O}C in ambient air for 24 hours. After the prescribed period of incubation, the zone of inhibition was measured using a Vernier caliper (against transmitted light) and the results interpreted using the CLSI (2022) guidelines.

The D-test

The D-test (CLSI, 2022) was used to detect the inducible MLSB (iMLS$_B$) phenotype by placing 15µg erythromycin and 2µg clindamycin disks (Oxoid, UK) 15mm apart. Plates were incubated at 35^{O}C in ambient air for 18 hours. After incubation, flattening of the zone of inhibition adjacent to the erythromycin disk (D-zone) indicated inducible clindamycin resistance. Isolates which expressed resistance to both clindamycin and erythromycin were interpreted as constitutive MLS$_B$ phenotype (iMLS$_B$), while those resistant to erythromycin but susceptible to clindamycin with no flattening of the zone of inhibition were considered macrolide streptogramin (MS) phenotype.

RESULTS AND DISCUSSION

In light of the increasing prevalence of drug-resistant organisms, the acquisition of precise identification and susceptibility test data from bacterial isolates has become crucial in order to make informed decisions regarding appropriate treatment strategies. Having accurate and reliable information allows healthcare professionals to select the most effective antibiotics and combat the challenges posed by drug resistance effectively. *Staphylococcus aureus* has been recognized for a considerable period of time as a significant pathogen in human diseases and holds the position as the primary cause of nosocomial (hospital-acquired) infections. Routine in vitro tests for clindamycin susceptibility may not provide accurate identification of iMLS$_B$ resistance caused by *erm* genes. This can result in ineffective treatment if the resistance goes undetected. Therefore, it is crucial to incorporate the D-test as a routine practice to effectively detect and address such resistance, ensuring appropriate treatment strategies are implemented. (Ghanbari *et al.,* 2016; Mama *et al.,* 2019; Pratibha and Kumar, 2021).

A total of 100 non-repetitive clinical isolates of *Staphylococcus aureus* were collected during the time of the study, between September 2021 and February 2022. Following standard bacteriological techniques, 49 (49%) were confirmed to be *Staphylococcus aureus*. Out of which MRSA (Figure 1) account for 44.9% (22/49). With GARSH having the highest number of misidentified isolates at 58%, followed by GHK at 51%, and TWCSH at 27% (Table 1). This rate is excessive for an isolate like *Staphylococcus aureus*.

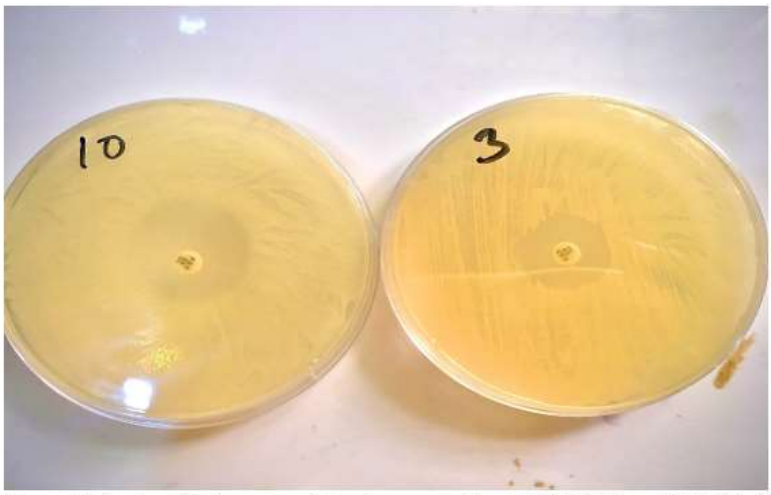

Figure 1 Mueller Hinton agar plates demonstrating MSSA (10) and MRSA (3)

Table 1. Sources and categorization of *Staphylococcus aureus* isolates

Hospital	Isolates re-characterized (%)	*Staphylococcus aureus* (%)
GHK	53(53)	26(53.1)
GARSH	36(36)	15(30.6)
TWCSH	11(11)	08(16.3)
Total	**100**	**49**

Key; GHK = General Hospital Katsina, GARSH = General Amadi Rimi Specialist Hospital, and TWCSH = Turai Umar Musa Yar'adua Women and Children Specialist Hospital

Based on the nature of the samples, it was found that wound swabs have the highest prevalence at 38.8% followed by urine at 22.4%, urogenital swabs at 16.3%, aspirates at 14.3%, blood and sputum at 4.1% each (Table 2). The high prevalence observed in wound swabs can be attributed to a combination of factors related to host susceptibility, bacterial virulence, and environmental factors (Byrd *et al.,* 2018), and this finding is in accordance to similar studies from within Nigeria and other regions of the world (Medugu *et al.,* 2021; Thapa *et al.,* 2021; Mahfouz *et al.,* 2023).

Table 2. Categorization of *Staphylococcus aureus* isolates based on sampling sites.

Specimen	MRSA (%)	MSSA (%)	Total
Wound swabs	11(57.9)	8(42.1)	19(38.8)
Urine	3(27.3)	8(72.7)	11(22.4)
Aspirates	2(25)	6(75)	8(16.3)
Urogenital swabs	2(28.6)	5(71.4)	7(14.3)
Sputum	2(100)	0(0)	2(4.1)
Blood	1(50)	1(50)	2(4.1)
Total	**22(44.9)**	**27(55.1)**	**49(100)**

Key; MRSA-Methicillin Resistant *Staphylococcus aureus* and MSSA-Methicillin Susceptible *Staphylococcus aureus*

D-test (Figure 2) results demonstrated that the prevalence of $cMLS_B$, MS, $iMLS_B$ and resistance phenotypes among *Staphylococcus aureus* isolates was 6.1%, 18.4% and 25% respectively (Figure 3), while 40.8% isolates were sensitive to both erythromycin and clindamycin. It's worth noting that; all the $iMLS_B$ and $cMLS_B$ phenotypes were observed to be MRSA isolates. The prevalence of $iMLS_B$ resistance varies by geographic regions and even between hospitals as previously reported, from 14.2% to 32.3%, in Nigeria and different parts of the world (Kumurya, 2015; Chika *et al.,* 2018; Mama *et al.,* 2019; Medugu *et al.,* 2021; Pratibha and Kumar, 2021; Mahfouz *et al.,* 2023).

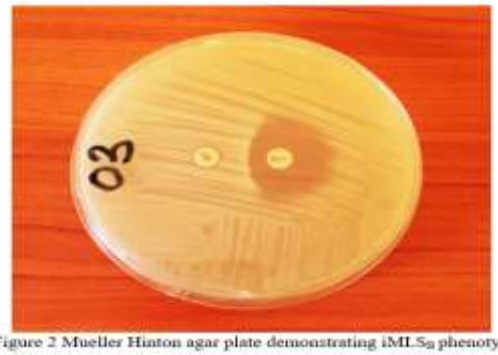

Figure 2 Mueller Hinton agar plate demonstrating $iMLS_B$ phenotype

Figure 3 Distribution of resistance pattern to MLS_B antibiotics. In this figure; iMLSB: inducible macrolide lincosamide-streptogramin B phenotype, MS: macrolide streptogramin phenotype cMLSB: constitutive macrolide-lincosamide streptogramin phenotype

However, results obtained from this study are similar to those obtained within Nigeria (Kumurya, 2015; Medugu *et al.,* 2021) and Ethiopia (Mama *et al.,* 2019). Some investigators have reported a higher incidence (Moosavian *et al.,* 2014) while others have indicated lower incidence (Chika *et al.,* 2018; Pratibha and Kumar, 2021 Mahfouz *et al.,* 2023). Without the D-test, around 1/4th of the erythromycin resistant isolates in this study could have been incorrectly identified as susceptible to clindamycin, leading to treatment failure.

CONCLUSION

The study primarily aimed to assess the frequency of inducible resistance to clindamycin using the D-test. To our knowledge, this study was the first of its kind to explore the prevalence of MLS_B resistant phenotypes in Katsina. The D-test emerged as a highly sensitive, user-friendly, and dependable method for identifying $iMLS_B$ strains within clinical laboratory settings, suggesting its suitability for routine implementation without requiring specialized testing facilities, and it's therefore recommended. The results of this study revealed that inducible macrolide-lincosamide-streptogramin B ($iMLS_B$) resistance was the prevailing resistance phenotype among the *Staphylococcus aureus* strains investigated. The findings further highlight the pressing need to enhance the quality of work in clinical laboratories, emphasizing the importance of accurate and reliable testing methodologies.

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