AJMAS
https://journal.utripoli.edu.ly/index.php/Alqalam eISSN 2707-7179

Research article

Phenotypic Detection of Macrolide, Lincosamide and Streptogramin B Resistance Among *Staphylococcus Aureus* Clinical Isolates in A Northern Nigeria Tertiary Hospital

Hassan Yahaya[1]*, Ahmad Saad Ahmad[1], Aminu Ibrahim[1], Sharif Alhassan Abdullahi[2]

[1]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University Kano, Nigeria*
[2]*Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, Bayero University Kano, Nigeria*

ARTICLE INFO

Corresponding Email: *hyahaya.mls@buk.edu.ng*
Received*: 22-03-2022* ***Accepted****: 30-03-2022* ***Published****: 01-04-2022*
Keywords*: Staphylococcus aureus, Clindamycin, Resistant, Strains, Antibiotics.*

ABSTRACT

Background and aims. *Staphylococcus aureus* **(***S. aureus***)** *is a frequently isolated pathogen associated with community and hospital infections. The emergence of Methicillin-Resistant S. aureus (MRSA) and macrolide–lincosamide–streptogramin B (MLSB) resistant strains pose a severe challenge to antibiotic selection. The study aimed to determine phenotypic MLSB resistant strains from clinical samples.* ***Methods****. Two hundred clinical samples from the wound, ear swab, urine, blood and sputum were collected. The occurrence of constitutive (cMLSB) and inducible (iMLSB) clindamycin resistance was phenotypically determined. A 31 non-duplicate, confirmed S. aureus were isolated and used. Antimicrobial susceptibility testing (AST) of the isolates was tested using six antibiotics; cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg) and cotrimoxazole (25 µg). The MLSB resistance phenotype of the isolates, erythromycin-resistant isolates were assessed by double-disk diffusion (D-test) for detection.* ***Results****. Of the 31 isolates, 21 (67.74%) were methicillin-susceptible Staphylococcus aureus (MSSA) and 10 (32.3%) were MRSA. Out of 21 MSSA strains, 5 were MLSB resistant phenotypes, of which 1 (4.8%) and 4 (19.0%) strains showed cMLSB and iMLSB resistance respectively. However, 6 out of 10 MRSA strains detected showed MLSB resistance. Both cMLSB and iMLSB resistance showed 3 (30%). The result showed that MRSA, cMLSB and iMLSB resistance occurs in clinical isolates of S. aureus from the study area.* ***Conclusion****. The use of a simple and cost-effective method (disk diffusion) for cefoxitin and the D-test for iMLSB organisms could easily identify resistant isolates. Antibiotic resistance profiles determination could optimize the therapy of multi-drug resistant strains.*

Cite this article: *Hassan Y, Ahmad S, Aminu I, Alhassan A Phenotypic Detection of Macrolide, Lincosamide and Streptogramin B Resistance Among Staphylococcus Aureus Clinical Isolates in A Northern Nigeria Tertiary Hospital. Alq J Med App Sci. 2022;5(1):193-198.* *https://doi.org/10.5281/zenodo.6406557*

INTRODUCTION

S. aureus is a human opportunistic pathogen responsible for superficial and life-threatening systemic infections, including septicemia, endocarditis and osteomyelitis. It often colonizes the skin, skin glands and mucous membranes, especially the anterior nares of healthy individuals, causing various clinical diseases [1]. The bacteria demonstrate continuous development and spread of resistance to a broad range of antimicrobial classes and can express different virulence factors, thus considered medically significant when present in clinical specimens [2]. *S. aureus* is one of the most frequently isolated pathogens in community and hospital-acquired infections and is also a causative agent of bacteremia. It causes severe infections in different tissues, including skin, soft tissue, surgical site infections, necrotizing fasciitis, gastroenteritis, and pneumonia [3,4].

Macrolides (e.g., erythromycin, azithromycin), lincosamides (e.g., clindamycin, lincomycin), and streptogramin B (e.g., quinupristin) are groups of antibiotics collectively termed MLSB. Resistance to MLSB antibiotics is associated with three main mechanisms: methylation of rRNA (target modification), active efflux and enzymatic inactivation [5]. The expression of resistant phenotypes to MLSB antibiotics can be constitutive (cMLSB) or inducible (iMLSB), encoded by erythromycin ribosome methylase (*erm*) genes. The MLSB antibiotics are commonly used in the treatment of staphylococcal infections [4]. Erythromycin and clindamycin are commonly used for the treatment of *S. aureus* infections. In penicillin-allergic

patients, clindamycin is a good alternative in the treatment of *S. aureus* infections. It is also frequently used to treat skin, soft tissue and bone infections because of its tolerability, cost, effectiveness, oral form and excellent tissue penetration (with exception of the central nervous system). It accumulates in abscesses and no renal dosing adjustment is required [5].
MLSB antibiotics have frequently been preferred in the treatment of infections caused by Gram-positive bacteria. Although they differ in their chemical structures, their mechanisms of action are similar. Therefore, genes causing resistance to any of the MLSB group of antibiotics may lead to cross-resistance to others [3, 6-8].
These genes encode enzymes for inducible or constitutive resistance to MLSB agents through methylation of the 23S ribosomal RNA. Thus, macrolides, lincosamides, and Type B streptogramins (MLSB resistance) are affected by reducing MLSB agents' binding to the ribosome [9-13]. *S. aureus* resistance to macrolide antibiotic may also be due to an active efflux mechanism, encoded by Methionine Sulfoxide Reductase A (*msrA*) gene, which encodes resistance to macrolides and Type B streptogramin only [5].
In vitro grown *S. aureus* isolates with constitutive resistance (cMLSB) were resistant to erythromycin and clindamycin. Those with inducible resistance (iMLSB) are resistant to erythromycin but appear to be susceptible to clindamycin [14]. Strains with inducible resistance to clindamycin are challenging to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive *in vitro* when these antibiotic discs are not placed adjacent to each other. In such a case, in vivo therapy with clindamycin may select only constitutive *erm* mutants leaving the inducible ones, thus, leading to a clinical therapeutic failure [15].
In case of resistance mediated through the *msrA* gene (i.e., efflux of antibiotics), staphylococcal isolates appear as erythromycin-resistant and clindamycin sensitive both *in vivo* and *in vitro*. The strain does not typically become clindamycin-resistant during therapy. These isolates are Macrolide-streptogramin (MS) phenotypes, and clindamycin can be safely administered in infections with these phenotypes with no risks of therapeutic failure [15-18].
The iMLSB resistant phenotypes are not identified using standard susceptibility test methods; instead, they are identified by erythromycin-clindamycin disc approximation test (D-test) and their resistant genes can be demonstrated by molecular methods [19]. Therefore, it is essential to detect such strains for the better outcome of patients on clindamycin therapy [20]. The study aimed to detect iMLSB resistant phenotypes among patients attending AKTH Kano, Nigeria.

METHODS

Bacterial growth and isolation

Two hundred (200) clinical samples including wound and ear swabs, urine, blood culture and sputum were aseptically collected from both inpatients and outpatients attending Aminu Kano Teaching Hospital, Kano State, Nigeria, between June-August 2021. The samples were processed and examined at the Microbiology Department of the Hospital. A total of 31 non-duplicated *S. aureus* isolates were recovered. The swabs were cultured on mannitol salt agar (MSA) (Merck, Germany) and incubated at 37 °C for 48 h for colony growth. Golden to round yellow colonies on MSA indicated mannitol fermentation associated with *S. aureus* (presumptive test). Colonies conforming with desired phenotypic characters on MSA with consistently positive results for Gram's stain reaction and biochemical tests (catalase test, coagulase test) were phenotypically confirmed as *S. aureus* [1,3].

MRSA identification

Both oxacillin (1 µg) and cefoxitin (30 µg) were used for the identification of MRSA isolates on Mueller-Hinton agar (MHA) plates following overnight incubation at 35 °C. *S. aureus* isolates that showed a zone of inhibition ≤21 mm with oxacillin (1 µg) and cefoxitin (30 µg) were considered MRSA according to Clinical and Laboratory Standards Institute (CLSI) guidelines [2,6].

Antibiotic susceptibility profile

The antibiotic susceptibility testing was performed based on the Kirby-Bauer disc diffusion method using MHA plates according to CLSI guidelines. Antibiotic disks (Oxoid, UK) including oxacillin (1 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), and chloramphenicol (30 µg) were used in the study.

Detection of constitutive and inducible MLSB resistance

Constitutive MLSB resistance is indicated in *S. aureus* isolates that showed no inhibition zone size or ≤13 mm around erythromycin and ≤21 mm around clindamycin. In contrast, inducible MLSB resistance is indicated as a flattening of the zone of inhibition around the clindamycin disc next to the erythromycin disc producing a 'D'-shaped zone of inhibition. The

result indicated that erythromycin had induced clindamycin resistance in the *S. aureus* isolates. Accordingly, a bacterial suspension equivalent to the turbidity of 0.5 McFarland standard was prepared and cultured on MHA to obtain a lawn growth. A clindamycin disk (2 µg) and erythromycin disk (15 µg) were placed 15 mm apart on an MHA plate. The plates were examined after incubation at 35° C for 24 h *S. aureus* ATCC 25923 was used as a standard control strain for disk diffusion assays.

RESULTS

The confirmed *S. aureus* isolates were further characterized for MRSA and MLSB. The antibiogram profile of the isolates was determined using six different antibiotics. Most of the *S. aureus* isolates (21) were recovered from wound swabs sample while sputum samples have the least number of the recovered *S. aureus* (Table 1).

A total of 31 isolates, 21 (67.74%) were susceptible to methicillin and 10 (32.26%) were methicillin while none of the isolates displayed moderate to cefoxitin, erythromycin and clindamycin. Most of the isolates, that is, 21 (83.87%) were sensitive to chloramphenicol, however, 19(61.28%) were resistant to clotrimazole (Table 2). Only 10 (67.7%) of the isolates were MRSA, while 21 (32.3%) isolates were MSSA (Table 3). Of the 21 MSSA strains, 5 (45.5%) were MLSB resistance phenotypes, of which 1 (4.8%) and 4 (19.0%) strains showed constitutive and inducible MLSB resistance, respectively (Table 3). On the other hand, 6 out of 10 MRSA strains showed MLSB resistance, of which 3 (30%) strains showed constitutive and 3 (30%) showed inducible MLSB resistance (Table 3) (Figure 1). The frequency of cMLSB resistant and iMLSB resistant was 4 (12.90%) and 7(22.58%) respectively, while 20 (64.52%) were MLSB susceptible (Table 4).

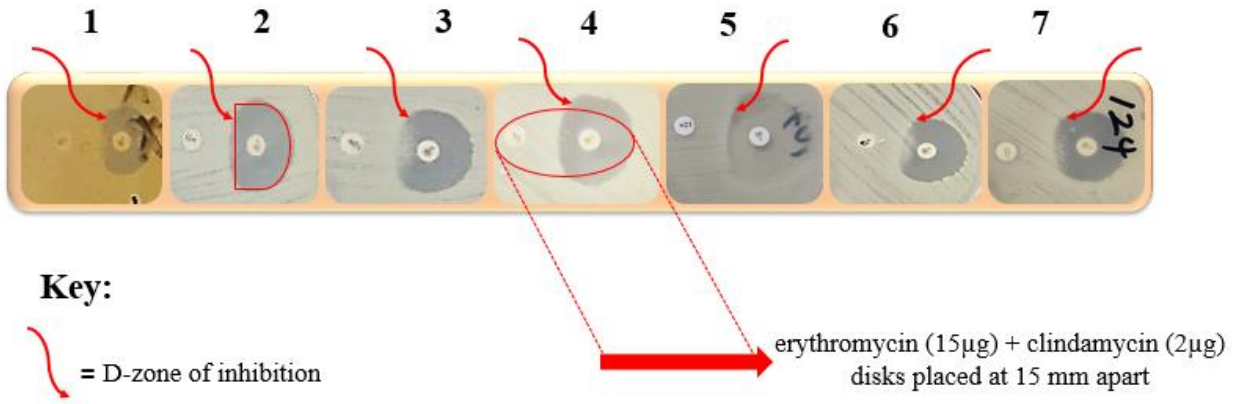

Figure 1: Inducible clindamycin resistance detected by disk diffusion method. Key: Isolates number: 1 = 15; 2 = 42; 3 = 47; 4 = 66; 5 = 107; 6 = 108; 7 = 124.

Table 1. Prevalence of S. aureus according to sample type

Sample type	Total No. of samples screened	Confirmed *S. aureus* isolates	% Prevalence
Wound	116	21	10.5
Ear swab	33	5	2.5
Urine	30	2	1.0
Blood culture	12	2	1.0
Sputum	9	1	0.5
Total	**200**	**31**	**15.5**

Table 2. Antibiotic susceptibility pattern of S. aureus isolates

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)	Total (%)
Cefoxitin (30 µg)	21 (67.74)	-----	10 (32.26)	31 (100)
Erythromycin (15 µg)	18 (58.06)	-----	13 (41.94)	31 (100)
Clindamycin (2 µg)	20 (64.52)	-----	11 (35.48)	31 (100)
Chloramphenicol (30 µg)	26 (83.87)	3 (9.68)	2 (6.45)	31 (100)
Ciprofloxacin (5 µg)	16 (51.61)	6 (19.35)	9 (29.04)	31 (100)
Cotrimoxazole (25 µg)	11 (35.47)	1 (3.25)	19 (61.28)	31 (100)

Table 3. Occurrence of constitutive and inducible MLSB resistance in S. aureus clinical isolates

Strain	N (%)	cMLSB (%)	iMLSB (%)	p-value
MSSA	21(67.7)	1 (4.80)	4 (19.0)	0.303
MRSA	10(32.3)	3 (30.0)	3 (30.0)	0.303
TOTAL	**31(100)**	**4 (34.76)**	**7 (49.0)**	

KEY: MSSA= Methicillin susceptible S. aureus; MRSA= Methicillin-resistant Staphylococcus aureus; MLSB= Macrolide, Lincosamide and Streptogramin B antibiotics; cMLSB= Constitutive MLSB; iMLSB= Inducible MLSB; Probability value (p-value) = 0.303 (>0.05)

Table 4. Overall prevalence of MLSB resistance in S. aureus isolates

Isolates characteristics	N (%)
cMLSB resistant	4(12.90)
iMLSB resistant	7(22.58)
MLSB susceptible	20(64.52)
TOTAL	**31(100)**

KEY: MLSB= Macrolide, Lincosamide and Streptogramin B; cMLSB= Constitutive MLSB; iMLSB= Inducible MLSB

DISCUSSION

Antimicrobial resistance is a worldwide problem linked to community and hospital-associated pathogens. Relevance of *Staphylococcus* species in both hospital and community-acquired infections is the utmost in controlling antibiotic resistance [6]. The emergence of multidrug-resistant bacterial strains occurs due to widespread and inappropriate administration of broad-spectrum antibiotics [7].

This study showed the existence of MLSB resistant *S. aureus* circulating in different communities in Kano. Resistance to MLSB drugs results in poor management of the affected patients. *S. aureus* is one of the leading bacterial pathogens that causes bacteremia and nosocomial infections. Increasing antimicrobial resistance in *S. aureus* has become a global phenomenon. The increasing prevalence of MRSA necessitates the new focus on the use of MLSB antibiotics to treat. *aureus* infections to avoid further emergence of resistance [6].

This study showed that the overall prevalence of *S. aureus* in the study area was 15.5%. The antimicrobial susceptibility testing showed that the *S. aureus* isolates had varying levels of susceptibility and resistance to the tested antibiotics. The isolates showed high levels of susceptibility to chloramphenicol (83.87%), cefoxitin (67.74%) and clindamycin (64.52%), while average susceptibility was shown in erythromycin (58.06%) and ciprofloxacin (51.61%). On the other hand, a high level of resistance was shown in cotrimoxazole (61.28%) which corroborates with the findings of Mohamed et al. [8] that reported ciprofloxacin (60%), however, contrary with erythromycin (90%) resistance. The susceptibility results of the clindamycin (64.52%) assay are also in agreement with the findings of Mohamed et al. [8].

In this study, the MRSA prevalence among the clinical isolates was 32.26% which may be considered high. The prevalence of MRSA obtained in this study was higher than the study conducted by Abdullahi and Iregbu [9] in Central Nigeria. The result was also higher than the findings of Okojokwu et al. [9] that reported 16.7% MRSA prevalence obtained from the

palms of the poultry farm workers in Jos, Central Nigeria. Perhaps the difference could be attributed to geographical location and the methodology approach.

In MRSA strains, the prevalence of cMLSB resistance was 27.30% and that of iMLSB resistance was also 27.30%, while in MSSA strains, the prevalence of constitutive MLSB resistance was 9.10% and that of inducible MLSB resistance was 36.40%. The MRSA and MSSA prevalence rates were lower than a study conducted by Raut et al. [2], which reported 70.0% and 55.9% for constitutive MLSB and inducible MLSB, respectively. The authors also reported 30.0% and 44.1% for constitutive MLSB and inducible MLSB, respectively, associated with MSSA isolates.

The overall prevalence of MLSB resistance in all the *S. aureus* isolates was 35.48%, of which 12.90% showed constitutive resistance, while 22.58% showed inducible resistance to clindamycin. The prevalence of iMLSB resistance observed in this study was comparable to the 17.7% prevalence in North Central Nigeria by Okojokwu et al. [10]. It could be suggested that the 22.58% of the isolates that showed inducible resistance to clindamycin could be falsely evaluated as susceptible to clindamycin by routine disc diffusion method if D-test had not been applied.

CONCLUSION

This study showed a high prevalence of MRSA, cMLSB and iMLSB among *S. aureus* clinical isolates in Aminu Kano Teaching Hospital, Kano, Nigeria. The disk diffusion using cefoxitin for MRSA and D-test for iMLSB detection is a cost-effective and straightforward method that can be used in resource constraint areas. It is important to note that the determined clindamycin susceptibility in clinical settings is critical in the proper management of patients infected with resistant *S. aureus.*

The prevalence of MRSA strains among the clinical isolates was 32.26%. The prevalence of MLSB resistant phenotypes in the *S. aureus* isolates was 35.48%, of which 12.90% showed constitutive resistance, while 22.58% showed inducible resistance to clindamycin. While both erythromycin and clindamycin are good antibiotics that interfere with the protein synthesis of their target bacterium, the presence of constitutive and inducible MLSB resistance could render these antibiotics ineffective for treatment. Treatment failure risks when clindamycin is used to treat infections caused by *S. aureus* strains carrying the inducible resistance gene (erythromycin ribosome methylase). The presence of MLSB resistance in MRSA poses a serious predicament in healthcare and community-associated transmissions.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

REFERENCES

1. Silva V, Hermenegildo S, Ferreira C, Manaia CM, Capita R, Alonso-Calleja C, et al. Genetic characterization of methicillin-resistant staphylococcus aureus isolates from human bloodstream infections: Detection of MLSB resistance. Antibiotics. 2020;9(7):1–9.
2. Raut S, Bajracharya K, Adhikari J, Pant SS, Adhikari B. Prevalence of methicillin resistant Staphylococcus aureus in Lumbini Medical College and Teaching Hospital, Palpa, Western Nepal. BMC Research Notes. 2017;10(1):1–7.
3. Zarizal S, Yeo CC, Faizal GM, Chew CH, Zakaria ZA, Al-Obaidi MMJ, et al. Nasal colonisation, antimicrobial susceptibility and genotypic pattern of staphylococcus aureus among agricultural biotechnology students in besut, terengganu, east coast of malaysia. Tropical Medicine and International Health. 2018;23(8):905–13.
4. Masiuk H, Wcisłek A, Jursa-Kulesza J. Determination of nasal carriage and skin colonization, antimicrobial susceptibility and genetic relatedness of Staphylococcus aureus isolated from patients with atopic dermatitis in Szczecin, Poland. BMC Infectious Diseases. 2021;21(1):1–9.
5. Ivanova N, Gugleva V, Dobreva M, Pehlivanov I, Stefanov S, Andonova V. We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. Intech. 2016;i(tourism):13.

6. Nashwa MA-K, Noha TAE-K. Phenotypic and genotypic detection of macrolide-lincosamide-streptogramin B resistance among clinical isolates of Staphylococcus aureus from Mansoura University Children Hospital, Egypt. African Journal of Microbiology Research. 2017;11(12):488–94.
7. Kępa M, Miklasińska-Majdanik M, Wojtyczka RD, Idzik D, Korzeniowski K, Smoleń-Dzirba J, et al. Antimicrobial potential of caffeic acid against staphylococcus aureus clinical strains. BioMed Research International. 2018;2018.
8. Mohamed SH, Elshahed MMS, Saied YM, Mohamed MSM, Osman GH. Detection of heavy metal tolerance among different mlsb resistance phenotypes of methicillin-resistant S. aureus (MRSa). Journal of Pure and Applied Microbiology. 2020;14(3):1905–16.
9. Abdullahi N, Iregbu K. Methicillin-Resistant Staphylococcus aureus in a Central Nigeria Tertiary Hospital. Annals of Tropical Pathology. 2018;
10. Okojokwu OJ, Akpakpan EE, Azi1 HY, Abubakar BS A-OJ. Inducible Clindamycin Resistance in Staphylococcus aureus Isolated from Palms of Poultry Workers in Jos, Plateau State, Nigeria. Journal of Infection and Molecular Biology; 2018.11–5.