

JOPAT Vol 24(1), 1879 – 1889. Jan – June, 2025 Edition.

ISSN2636 – 5448

<https://dx.doi.org/10.4314/jopat.v24i1.12>

PHENOTYPIC AND GENOTYPIC CHARACTERISATION OF METHICILLIN AND INDUCIBLE CLINDAMYCIN CO-RESISTANT *Staphylococcus aureus* ISOLATED FROM CLINICAL SAMPLES

Achukwu, Ngozika O*, Ugbaja, Chinaza M, Ugbajah Winston C and Achukwu, Peter. U.

Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus.

Abstract

The increase in microbial resistance to antibiotics is emerging as a challenge globally in the treatment of infections. The emergence of multiple drug-resistant microbial infections has created a severe concern for public health. Phenotypic and genotypic estimation of these resistant genes will assist in mitigating measures to curb the rising incidence. The aim of this study is to isolate and characterize inducible clindamycin and methicillin co-resistant *Staphylococcus aureus* from clinical samples phenotypically and genotypically. Clinical samples collected from the various hospitals were cultured on mannitol salt agar, blood agar and incubated overnight at 37°C. Colonial morphology, API identification system and standard identifications including Gram stain reaction, catalase, and coagulase were used to identify the isolates. The antibiotic susceptibility and D tests were carried out on Mueller- Hinton agar using the modified Kirby-Bauer method according to the guidelines of the Clinical Laboratory Standard Institute (CLSI). The overall occurrence of *S. aureus* was 75% (160/214). Multiple drug-resistant, *S. aureus* was 50% (81/160), methicillin resistance was 32%, (51/160), and inducible clindamycin resistance was 29.38% (47/160). The prevalence of co-resistance of inducible clindamycin and Methicillin resistance was 23.75%. The prevalence of resistance genes among the isolates with co-resistance of inducible clindamycin and methicillin resistance genes were 13.7%, 17.6, 5.9, 5.9, for Mec A, Mec C, Erm B and Erm C respectively. The highest incidence of inducible clindamycin and methicillin co-resistance was seen in urine samples. This study reveals a high level prevalence of methicillin and inducible clindamycin co- resistance among the isolates from the clinical samples studied which is a public health threat.

Keywords: D-test, clindamycin, Resistance, *Staphylococcus aureus*, Erythromycin, Methicillin

Corresponding author: ngozika.achukwu@unn.edu.ng

Introduction

Multiple drug resistance has been on the increase in the past decade and this has caused treatment failures and increased economic burden especially in developing countries [1], because the commonly used antibiotics for treatment are no longer effective [2]. The burden is more among the West African Countries With an estimated 27.3 deaths in every 100,000 linked to antimicrobial resistance [3]. This could be attributed to a number of factors such as rampant distribution of fake and substandard drugs, poor diagnostic procedures, inadequate diagnosis before treatment, failure to adhere to treatment regimen, easy access to drugs without proper prescription, unrestricted access of the public to antibiotics over the counter, poor drug monitoring and surveillance [4-5]. Therefore there is an urgent need for immediate intervention in all health sectors to fight against this pandemic through surveillance. . Proper prescription may not be an effective means of limiting access to drugs particularly in sub-Saharan Africa where persons with and without proper prescription have access to drugs over the counter [2].

Thirty percent of healthy individuals harbor *Staphylococcus aureus* as part of the normal flora. [6]. *S. aureus* can be found on the skin, mucous membranes, nasal passages, throat, intestines, and mouth [7 - 8]. Across the world, *Staphylococcus aureus* is a foremost contributor to community-acquired and nosocomial infections [9]. *S. aureus* can cause broad range of serious infections, ranging from soft tissue and mild skin infections, to potentially fatal systemic diseases like food poisoning, meningitis, scalded skin syndrome, pneumonia, endocarditis and bloodstream infections. These infections are linked to a significant morbidity and mortality rate [10]. Multiple drug resistance has emerged as a result of the widespread use of antibiotics, which has raised the selective pressure on microorganisms. These resilient bacteria are cause for concern to public health worldwide [11]. Antimicrobial drugs become less effective when resistant isolates proliferate, leading to longer hospital admissions, higher

treatment costs, and a rise in mortality [12]. Macrolide lincosamide-streptogramin B (MLSB) antibiotics class is a potential substitute for the treatment of *staphylococcus aureus* infections, as reported resistance to most antibiotics used to treat these infections is on the rise [12]. Clindamycin belongs to the MLSB family and is frequently employed as an optimal antibiotic due to its superior pharmacokinetics [13] However, a greater percentage of Staphylococcal strains are becoming resistant to the MLSB family of antibiotics as a result of their extensive use and misuse [14]. The purpose of this study was to identify and characterize co-resistance of inducible Clindamycin (iMLSB) and methicillin resistance of multiple drug resistant *S. aureus* from clinical samples and their possible resistant markers.

The antibiogram generated from this study will assist in providing accurate data on antibiotic susceptibility testing to aid therapeutic decision making.

Materials and Methods

Study area

This study was carried out in Department of Medical Laboratory Science University, of Nigeria Enugu Campus. All samples (urine, CSF, blood, swabs and other body fluids were collected from patient presenting at University of Nigeria teaching Hospital that came for microbiological examination who gave consent within the period of September, 2023 - March, 2024). A total of 214 samples were collected.

Inclusion/ Exclusion criteria

Isolates obtained from clinical specimen of both genders were used in this study. Only one sample was collected from patients that were to submit more than one sample.

Laboratory Investigations

All the samples collected were cultured on Mannitol salt agar and blood agar by streaking method to obtain discreet colonies using the method described by clinical and laboratory standard institute (CLSI) [15] and incubated overnight aerobically at 37°C. Cultural morphology, Gram reaction, catalase, coagulase and API testing system (API STAPH) inbuilt

protocol of Biomerieux laboratory for identification as described by the manufacturer were performed to confirm *S. aureus* used in this study. The resistant profiles of the isolates were determined using the following antibiotics ceftriaxone, erythromycin, gentamycin, ampicillin, clindamycin, levofloxacin, cefoxitin, Oxacillin, ceftraoline and trimethoprim-sulfamethoxazole.

Kirby-Bauer agar diffusion method was used as described by CLSI [15] for the susceptibility testing and the results interpreted as resistant, intermediate and sensitive according to the CLSI recommended inhibition zone diameter to the nearest mm. The isolates were sent for molecular studies for detection of resistant genes using multiplex PCR.

Methicillin resistant *Staphylococcus aureus* (MRSA) Phenotypic Detection

Cefoxitin (30 µg) and oxacillin (1 µg) were used in detecting the phenotypic classification of the *Staphylococcus aureus* into MRSA and MSSA as described by CLSI [15]. A standardized bacteria suspension was cultured on Muller Hinton agar, the Cefoxitin and Oxacillin disc placed on it. Cefoxitin disc with an inhibition zone diameter less than 21mm is reported as resistant while greater than 22mm is reported as sensitive. Oxacillin disc with an inhibition zone diameter less than 21mm is reported as resistant

D-Test procedure for phenotypic detection of iMLSB

D- Test procedure as described by CLSI [15] was used. Standardized bacterial suspension was prepared and the turbidity adjusted to 0.5 McFarland's. It was used to inoculate a sterile Muller–Hinton agar plate

(MHA). Erythromycin (15 µg) and clindamycin (2 µg) set 15mm apart and were incubated at 37°C for 24 hours. The zones of inhibition diameter were read and the phenotypic appearance observed after 24 hours of incubation.

Interpretation of the D-Test

Isolates with zones of inhibition diameter with erythromycin -R = ≤13 mm; Erythromycin -I = 14 to 22 mm, erythromycin -S = ≥23 mm, while clindamycin-R = ≤14 mm clindamycin -I = 15 to 20 mm, clindamycin -S = ≥21 mm. If the erythromycin zone is ≤13 mm and the clindamycin zone is ≥21 mm. D-test positive is zone of inhibition ≥21 mm clindamycin and erythromycin ≤13 mm with a D-shaped halo around the clindamycin, common phenotypes include

- (1) iMLSB (Inducible clindamycin resistance phenotype): Resistant to erythromycin but produce a D-shaped zone of inhibition around the clindamycin
- (2) cMLSB (Constitutive clindamycin resistance phenotype) Resistant to both clindamycin and erythromycin
- (3) MS phenotype (Macrolide streptogramin) Sensitive to clindamycin but resistant to erythromycin
- 4) S phenotype: (Sensitive) Sensitive to both clindamycin and erythromycin.

Genetic detection of resistance genes

Isolates that demonstrated phenotypic presence of inducible clindamycin and MRSA genes were screened for genetic presence for resistance genes of MRSA, clindamycin and erythromycin resistance genes. The DNA extraction was done as described by [5].

Table 1: Primers used for the resistant gene identification

Genes	5' -3' primer	Amplicon size (bp)	Reference
<i>Erm A</i>	F:TATCTTATCGTTGAGAAG GGATT R:CTACACTTGGCTTAGGAT GAAA	139	Agbo et al. [5]
<i>Erm B</i>	F:CTATCTGAT TGTTGAAGA AGGATT R:GTTTACTCT TGGTTTAGG ATGAAA	142	Agbo et al. [5]

<i>Erm C</i>	F:CTTGTTGAT CACGATAAT TTCC R:ATCTTTTAG CAAACCCGT ATTC	190	Agbo et al. [5]
<i>Mec A</i>	F:AGTTCTGCA GTACCGGAT TG R:AAAATCGA TGGTAAGGT TCGC	533	Idrees, et al., [16]
<i>Mec C</i>	F -TCACCAGGTTCAAC[Y]C AAAA- R-CCTGAATC[W]GCTAATAA TATTC-	356	Idrees, et al., [16]

Ethical Approval

Ethical permission to carry out this work was sought from the Ethics Committee College of Medicine University of Nigeria Enugu Campus.

Results

Distribution of *Staphylococcus aureus* in clinical specimen

The isolates that produced yellow colonies on mannitol salt agar, exhibited a clear zone of hemolysis on blood agar, and tested positive for catalase and coagulase were further identified using the API system and confirmed as *Staphylococcus aureus*.

Out of the 214 isolates collected, 75% (160/214) were confirmed to be *Staph aureus* while 50.6% (81/160) were multiple drug resistant and 36% (51/160) were methicillin resistant with 68.12% (109/160) MSSA as shown in Fig 1

The isolates confirmed to be *S. aureus* (160/21) were obtained from various clinical specimen as shown in Fig 2 and its frequency were as follows: blood (5, 3.1%), CSF (10, 6.3%), ear swab (11, 6.9%), HVS (35, 21.3%), semen (17, 10.6%), ECS (3, 1.9%), urethral swab (11, 8.1%), urine (40, 25%), and wound swabs (28, 17.5%).

160 isolates of *S aureus* were used to perform the D- test and it was observed that 42.5% (68/160) express cMLSB phenotype, 29.4%, 47/160) expressed iMLSB phenotype, 15%, (24/160) express MS phenotype and 3.1%, (21/160) were both sensitive as shown in Fig 3

A total of (51/160) isolates presented phenotypic resistance to methicillin (only) and 47(29.37) inducible clindamycin (only) while 26.9% (43/160) demonstrated co-

resistance of inducible clindamycin and methicillin resistance as shown in Table 2. The highest co-resistance of inducible clindamycin and methicillin resistance was seen in isolates from urine and wound samples and the least from Endo Cervical Smear

The isolates that demonstrated positive resistant phenotypes were accessed for the presence of *MecA*, *Mec C*, *Erm A*, *Erm B* and *Erm C*, genes. From the PCR result it was observed that out of the 51 confirmed MRSA phenotypically 17.65% (11/51) has *Mec A* (533bp) genes but not detected in 78.4%, (40/51) and 25.5% (13/51) has *Mec C* (356bp) genes and not seen in 74.50%, (38/51) although all the 51 isolates were resistant to cefoxitin and oxacillin. Only 7.8% (4/51) has both *mecA* and *mecC* genes. *MecA* genes were detected more than *mecC* genes as shown in Fig 5

The isolates were also screened for macrolide resistance primary markers, The *Erm B* (142bp) gene was detected in 5.8%, (3/51), and *Erm C* (190 bp) genes were detected in 7.8% (4/51) while *Erm A* genes (139 bp), 0%, (0/51) was not detected in the isolates. Among the isolates with co-resistance of inducible clindamycin and methicillin resistance phenotypes 13.7%, 17.6%, 5.9%, 5.9%, harbour *mecA*, *mecC*, *Erm B* and *Erm C* respectively as shown in Fig 6

Antimicrobial Phenotypic and Genotypic resistance distribution pattern of *S. aureus* isolated from clinical samples as shown in table 4 reveals the types of phenotypic and genotypic resistant patterns from each of the clinical sample types. Urine and wound samples have the highest patterns of both phenotypes and the least is obtained in Urethra smear.

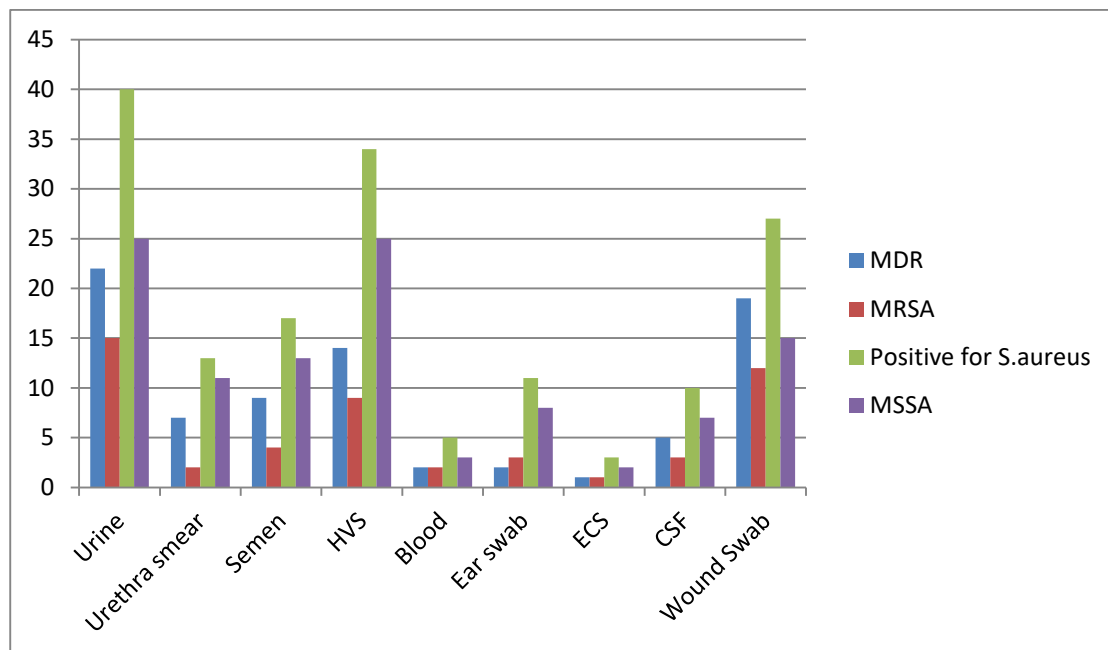
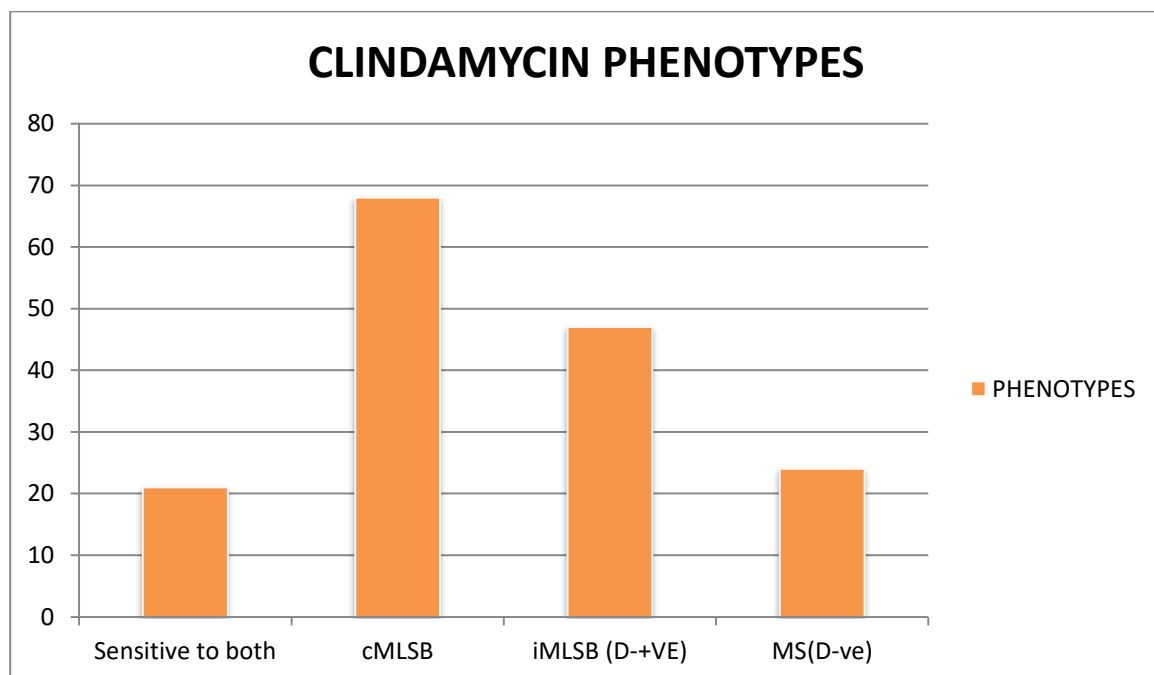
Fig 2: Distribution of *Staphylococcus aureus* in the clinical samplesFig 3: Clindamycin resistance *Staph aureus* phenotypes observed

Table 2: Phenotypic demonstration of co-resistance of inducible clindamycin and MRSA.

CLINICAL SAMPLE	iMLSB	MRSA	iMLSB + MRSA
Urine	22	15	15
Urethra swab	-	2	-
Wound swab	7	12	7
Semen	4	4	4
HVS	3	9	3
Blood	2	2	2

Ear swab	3	3	3
ECS	1	1	1
CSF	6	3	3

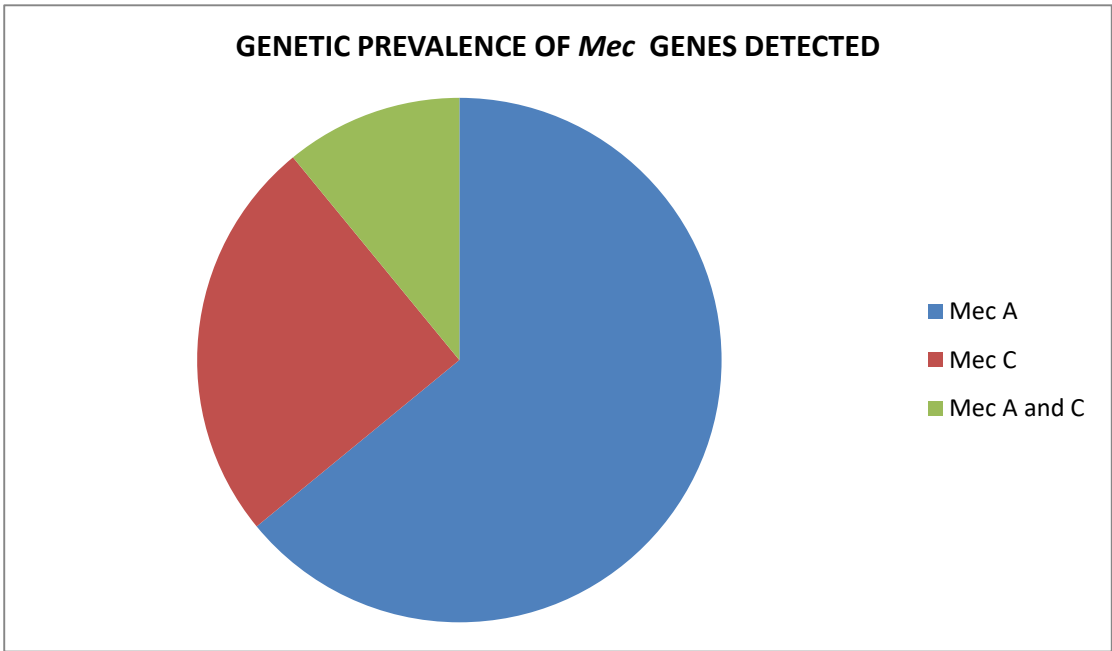


Fig 5: GENETIC PREVALENCE OF *mecA*, *mecB* AND *mecA* plus *mecB* GENES

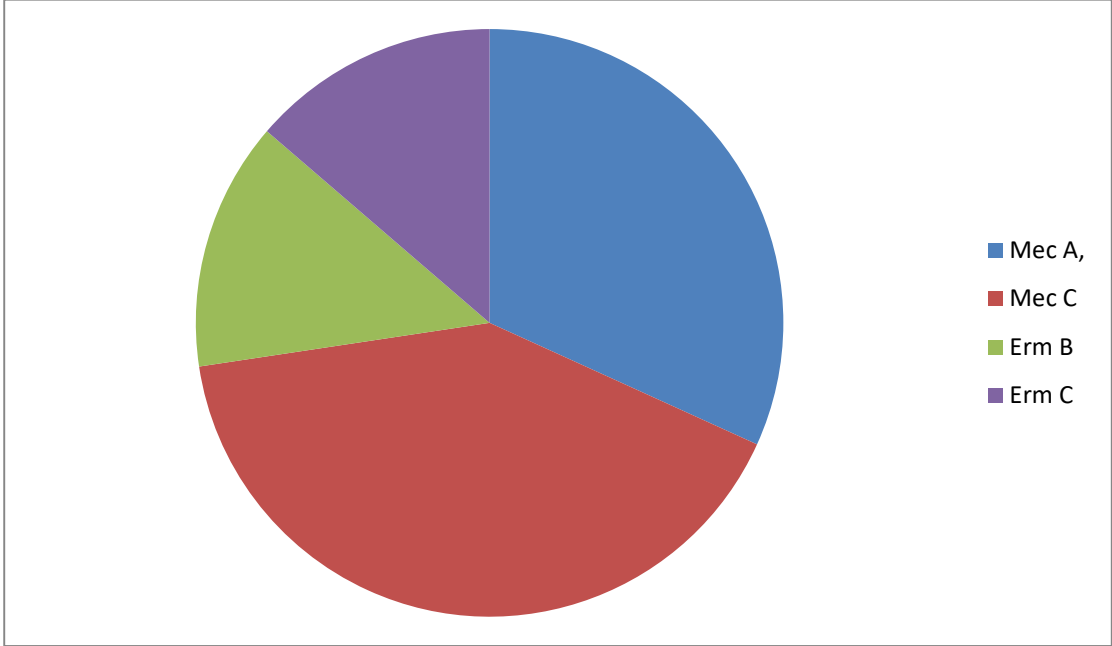


Fig 6: Prevalence of resistance genes among isolates with co-resistance of inducible clindamycin and methicillin resistance phenotypes

Table 3: Antimicrobial phenotypic and genotypic resistance distribution of *S. aureus* isolated from clinical samples

Clinical sample	Phenotypic	Genotypic
-----------------	------------	-----------

Urine	iMLSB, cMLSB, MS, S, MRSA	Mec A, Mec C, Erm B, Erm C
Urethra swab	MRSA	Mec A,
Wound swab	iMLSB, cMLSB, MS, S, MRSA	Mec A, Mec C, ErmB, Erm C
Semen	iMLSB, cMLSB, MS, S, MRSA	Mec A
HVS	iMLSB, cMLSB, MS, S, MRSA	Mec A, Mec C,
Blood	iMLSB, cMLSB, MRSA	Mec A
Ear swab	iMLSB, cMLSB, MS, S, MRSA	Mec A
ECS	iMLSB, cMLSB, MS, S, MRSA	Mec A
CSF	iMLSB, cMLSB, MRSA	Mec A

Key: iMLSB (Inducible resistance), **S** (Sensitive to both), **MS** (Resistance to both), **cMLSB** (Constitutive resistance),

Discussion

S. aureus is among the microbes that are implicated in multiple drug resistance because they can easily develop resistance to a wide range of antibiotic class with little treatment option [17]. The overall occurrence of *S. aureus* from this study was 75% (160/214). This is similar to the prevalence recorded by Agbo *et al.*, [5] (71.4%) and Nwaneri [18]. The prevalence of MRSA recorded was 36%. This prevalence is higher than the works of Ambachew, *et al.*, [6] (17%), Al-Humaidan, *et al.* [19] (25%), but lower than that recorded by Agbo *et al.*, [5] (47.3%) the discrepancy could be as a result of samples used and the sample size used in this study. The prevalence of MSSA is 68.1% which is lower than the works of [20], (79.3) and [6] (78.8%), [18] (89%), the variation could be linked to the use drugs in the locality, infection control and prevention methods being practiced in the area, the samples used and unrestricted prescription of drugs in the area. The swift spread of *S. aureus* strains resistance to antibiotics has complex the treatment options, mainly in middle-income countries and low - income countries like Nigeria.

In this study, the highest occurrence of *S. aureus* was seen in urine 40(25%), followed by HVS 35, (21.3%) then wound swab 28, (17.5%) and semen 17, (10.6%). this is contrary to report of [21] who reported wound swab and ear swab to have the highest occurrence while Urine and HVS had the least [21] Similar study recorded that greater part of the isolates were recovered from urine sample (76%) [22- 23] In this study, most of the *Staph aureus* 50.6%, (81/160) were multiple drug resistance and the MAR index is greater

than 0.2 which is indicative it's from a high risk source. The highest susceptibility was to ceftriaxone 133 (83.1%), followed by gentamycin 129 (80.6%) and ampicillin 121 (75.6%). The highest resistance was seen in clindamycin 82 (51.3%), followed by levofloxacin 81(50.6%) and erythromycin (65%). The increased resistance could be attributed to previous exposure of the isolates to these drugs which may have encouraged the development of resistance. This also could be linked to abuse and misuse of antibiotics in the area as a result of nonprescription, easy access to the drugs over the counter, inadequate treatment due to failure to complete drug doses and increase of fake and substandard drug and nearness of the locality to major hospitals and laboratories where the samples were obtained. This similar to reports of [5, 18]

Erythromycin is among the affordable antibiotics available in the locality erythromycin resistant rate of 65% in this study is actually worrisome due the fact that it acts as an inducer. Clindamycin is a lincosamide used in treatment of soft tissue and skin infection and also used as substitutes for patients who are affected by to penicillin [24]. The susceptibility rate of clindamycin in this study was 48.75% and its resistance rate was 51.3%. This drastic increase in resistance rate of clindamycin could be linked to the excessive and wrong use of clindamycin which is readily available over the counter. Therefore, there is an urgent need for the re-evaluation of antibiogram of *S. aureus* for a better understanding of its emerging and re-emerging pattern Treatment failure is recorded when strain carrying inducible erm gene is treated using non-inducer

macrolide or clindamycin [12]. Routine, In-vitro testing to detect inducible resistance is advocated to avoid failure in treatment [5], by performing D- Test on a routine basis. Out of 160 *S. aureus* isolated used to conduct the D-Test, 47(29.37%) demonstrated iMLSB phenotype, when compared with similar studies [6] recorded a lower rate of inducible clindamycin .25.7% and higher value was recorded by [13] (61%). This inconsistency may be as a result of number of samples used and the availability of clindamycin in the area of the study. The D-Test is the gold standard, reliable and a simple method for detecting iMSLB resistance to clindamycin. Recording *S. aureus* as been sensitive to clindamycin without performing D-test to determine the iMSLB status will led to failure in treatment

The occurrence of *mecA* gene from isolates in this study from clinical specimen is 17.6% and *mecC* 25.5%, this is alarming and a serious health threat in healthcare setting. This could be attributed to accumulative genetic effect of MRSA. This is similar to the works of [5] were *mecA* and *mecC* were isolated from *S. aureus* from clinical samples. Most of the isolates do not have any of the *mec* genes tested even though they demonstrated phenotypic resistance to methicillin. It is therefore very important to confirm any resistance using molecular method for any phenotypic resistance as only phenotypic detection tends to give a higher rate of resistance which could be as a result of the presence of other genes inducing the resistance. The occurrence of *MecC* genes recorded is higher than that of *mecA* genes this is similar to the woks of [25], but in contrast to the works of Idrees *et al.*, [16] were *mecA* were more prevalent than *mecC*. The occurrence of both *mecA* and *mecC* genes recorded in this study was 7.8%. This is less than that reported by [16] (51.7%).

The high prevalence of co-resistance of inducible clindamycin and methicillin resistance 23.75% is an indication of indiscriminate use and misuse of those antibiotics in the environment and this is confirmed by the presence of the resistance genes detected by PCR. Among the isolate

with co-resistance of inducible clindamycin and methicillin resistance genes 13.7%, 17.6, 5.9, 5.9, had *mecA*, *mecC* *ermB* and *ermC* respectively as shown in fig 6. Relevant authorities and the general public should work hand in hand to help bring a drastic stop to these super bugs by improving on sanitation and putting policies that will curtail the access to vital drugs without proper prescription. Due to lack of research on the co- resistance of inducible clindamycin and methicillin resistance *S. aureus* it is difficult to compare this prevalence with other works.

Conclusion

This study recorded a significant occurrence of *S. aureus*, with an increased number of iMLSB resistance among the *S. aureus* isolates, which is more in urine specimen. The increasing rate of antimicrobial resistance has resulted to treatment failure and consequently death. High co-resistance of inducible clindamycin and methicillin resistance was detected in this study. Detection of inducible clindamycin and methicillin resistance can contribute as one of the mitigating factors towards treatment failures. D-test should be incorporated routinely in our laboratory prior to prescription and administration of clindamycin. It is also important to genetically confirm the presence of any phenotypic detection of resistant gene

Acknowledgment

The authors wish to thank all the patients whose samples were used for this work. May God bless you all.

Source of funds: Self-funded

Conflict of interest disclosure

We declared no conflict of interest; the manuscript is original and has not been published by another journal.

Contribution of Authors

This work was conducted and approved in collaboration between all the authors, who take responsibility for its accuracy and integrity. Achukwu N.O designed the study; Ugbaja C.M and Ugbajah W. C

wrote the protocol; and contributed to the literature search; Achukwu N.O, Ugbaja C.M, Ugbajah W.C and Achukwu, P.U did lab experiments; Ugbaja C.M did the statistical analysis; Achukwu N.O, Ugbaja W.C and Achukwu, P.U Contributed in discussions; Ugbaja C.M, drafted the manuscript; Achukwu, P.U, supervised the study; Achukwu N.O wrote the final manuscript; Achukwu N.O, proofread the final manuscript for publication.

References

- [1] National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Population Health and Public Health Practice; Committee on the Long-Term Health and Economic Effects of Antimicrobial Resistance in the United States; Palmer GH, Buckley GJ, editors. Combating Antimicrobial Resistance and Protecting the Miracle of Modern Medicine. Washington (DC): National Academies Press (US); 2021 Oct 20. 3, The Health and Economic Burden of Resistance. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK577288/>
- [2] Kelly, T. K. W., Millar, B. C., and Moore, J. E. (2023). Antimicrobial Resistance (AMR). *British Journal of Biomedical Science*, 80, 11387. <https://doi.org/10.3389/bjbs.2023.11387>
- [3] Financial Times Limited. Antibiotic resistance in Africa: ‘a pandemic that is already here’. Available from: <https://www.ft.com/content/95f150df-5ce6-43cf-aa8d-01ac3bdcf0ef>
- [4] Chen, Q., Li, D., Beiersmann, C., Neuhaus, F., Moazen, B., Lu, G., and Müller, O. (2021). Risk factors for antibiotic resistance development in healthcare settings in China: a systematic review. *Epidemiology and infection*, 149, e141. <https://doi.org/10.1017/S0950268821001254>
- [5] Agbo, M.C., Ezeonu, I.M., Onodagu, B.O. Emencheta, S. C. Ezech C.C. (2024). Antimicrobial resistance markers distribution in *Staphylococcus aureus* from Nsukka, Nigeria. *BMC Infect Dis* 24, 320 (2024). <https://doi.org/10.1186/s12879-024-09126-1>
- [6] Ambachew, A., Gebrecherkos, T., and Ayalew, G. (2022). Prevalence and Clindamycin Resistance Profile of *Staphylococcus aureus* and Associated Factors among Patients Attending the University of Gondar Comprehensive Specialized Hospital, Gondar, Northwest Ethiopia. *Interdisciplinary Perspectives on Infectious Diseases*, 2022. 2(5), 17-2 <https://doi.org/10.1155/2022/6503929>
- [7] Elisa J M Raineri, Dania Altulea, Jan Maarten van Dijk, (2022). Staphylococcal trafficking and infection—from ‘nose to gut’ and back, *FEMS Microbiology Reviews*, 46(1), January, fuab041, <https://doi.org/10.1093/femsre/fuab041>
- [8] Sollid J. U. E., Furberg, A. S., Hanssen, A. M., and Johannessen, M. (2014). *Staphylococcus aureus*: determinants of human carriage. *Infection, genetics and evolution*, 21, 531-541
- [9] Goudarzi, M., Kobayashi, N., Dadashi, M., Pantůček, R., Nasiri, M. J., Fazeli, M., Pouriran, R., Goudarzi, H., Miri, M., Amirpour, A., and Seyedjavadi, S. S. (2020). Prevalence, Genetic Diversity, and Temporary Shifts of Inducible Clindamycin Resistance *Staphylococcus aureus* Clones in Tehran, Iran: A Molecular–Epidemiological Analysis From 2013 to 2018. *Frontiers in Microbiology*, 11, 493144. <https://doi.org/10.3389/fmicb.2020.00663>

- [10] Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., and Fowler Jr, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiology reviews*, 28(3), 603-661.
- [11] Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., and A. Alqumber, M. A. (2023). Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare*, 11(13). <https://doi.org/10.3390/healthcare11131946>
- [12] Thapa, D., Pyakurel, S., Thapa, S. (2021). *Staphylococcus aureus* with inducible clindamycin resistance and methicillin resistance in a tertiary hospital in Nepal. *Trop Med Health* 49, 90- 99
- [13] Adhikari R. P., Shrestha S., Barakoti A., Amatya R (2017). Inducible clindamycin and methicillin resistant *Staphylococcus aureus* in a tertiary care hospital Kathmandu Nepal. *BMC Infectious Diseases*. ;17(1): p. 483. doi: 10.1186/s12879-017-2584-5
- [14] Khoshnood, S., Shahi, F., Jomehzadeh, N., Montazeri, E. A., Saki, M., Mortazavi, S. M., and Maghsoumi-Norouzabad, L. (2019). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among methicillin-resistant *Staphylococcus aureus* strains isolated from burn patients. *Acta Microbiologica et Immunologica Hungarica*, 66(3), 387-398.
- [15] Clinical Laboratory Standards Institute. (2023). Performance standards for antimicrobial disk susceptibility tests; Approved standard-Clinical Laboratory Standards Institute 9th ed. CLSI document M2-A9. Wayne, Pennsylvania, USA: Clinical Laboratory Standards Institute; 2023
- [16] Idrees, M. M., Saeed, K., Shahid, M. A., Akhtar, M., Qammar, K., Hassan, J., Khaliq, T., and Saeed, A. (2023). Prevalence of *mecA*- and *mecC*-Associated Methicillin-Resistant *Staphylococcus aureus* in Clinical Specimens, Punjab, Pakistan. *Biomedicine*, 11(3), 878. <https://doi.org/10.3390/biomedicine11030878>
- [17] Guo Y., Song G., Sun M., Wang J., Wang Y. (2020). Prevalence and Therapies of antibiotic-resistance. *Frontiers in Cellular and Infection Microbiology*; 10 doi: 10.3389/fcimb.2020.00107.
- [18] Nwaneri, Miriam & Anejionu, Obinna and Ugo, Udochi. (2023). Investigation and mapping of the prevalence of “superbugs” Methicillin-resistant *Staphylococcus aureus* (MRSA) and antimicrobial susceptibility pattern in Enugu metropolis in Southeast Nigeria. *GSC Biological and Pharmaceutical Sciences*. 23. 245-254. 10.30574/gscbps.2023.23.3.0239.
- [19] Al-Humaidan O. S., El-Kersh T. A., Al-Akeel R. (2015). A. Risk factors of nasal carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* among health care staff in a teaching hospital in central Saudi Arabia. *Saudi Medical Journal*, 36(9):1084–1090. doi: 10.15537/smj.2015.9.12460.
- [20] Dilnessa T., Bitew A. (2016). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at yekatit 12 hospital medical college addis Ababa, Ethiopia. *BMC Infectious Diseases*. 2016;16(1): p. 398. doi: 10.1186/s12879-016-1742-5

- [21] Muhammad A. (2019) Prevalence of *Staphylococcus* Species from Clinical Samples Obtained from Some Hospitals on Kano Metropolis, Nigeria. *Am J Biomed Sci & Res.* 5(3). AJBSR.MS.ID.000913. DOI: 10.34297/AJBSR.2019.05.000913.
- [22] Chukwueze, C. M., Udeani, T. K., Obeagu, E. I., Ikpenwa, J. N and Nneka, A. (2022). Prevalence of Methicillin Resistant *Staphylococcus aureus* Infections among Hospitalized Wound Patients from Selected Tertiary Hospitals within Enugu Metropolis. *Journal of Advances in Medical and Pharmaceutical Sciences*, 24(3), 18-27.
- [23] Anejionu, M., Oli, A., Ezeudu, C., Ezejiofor, O., Ezeogu, J, Attama, A. and Okore, V. (2022) Methicillin-Resistant *Staphylococcus aureus* May Also Be Resistant to Clindamycin and Vancomycin. *Journal of Biosciences and Medicines*, **10**, 1-13. doi: [10.4236/jbm.2022.108001](https://doi.org/10.4236/jbm.2022.108001).
- [24] Armillei MK, Lomakin IB, Del Rosso JQ, Grada A, Bunick CG. (2024). Scientific Rationale and Clinical Basis for Clindamycin Use in the Treatment of Dermatologic Disease. *Antibiotics* .;13(3):270.<https://doi.org/10.3390/antibiotics13030270>
- [25] Kırmusaoğlu S. (2017). The mechanism of methicillin resistance and the influence of methicillin resistance on biofilm phenotype of *Staphylococcus aureus*. In: Enany S., editor. *The Rise of Virulence and Antibiotic Resistance in Staphylococcus aureus*. IntechOpen; London, UK: 25–41.