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Phenotypic and genotypic characterization of macrolide resistance in *Staphylococcus aureus* isolates from wound infection

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***Abstract*---**Microbiologists are increasingly concerned about the rise in *S. aureus* MLS_B (macrolide, lincosamide, streptogramin B) drug resistance. Clindamycin has been effective in treating infections by *S. aureus*, and the variations in clindamycin sensitivity patterns cause treatment to fail. Inducible clindamycin resistance in *S. aureus* is expressed via erythromycin ribosome methylase genes. In the current study, 25 *S. aureus* isolates were identified by conventional chemical tests and the Vitek®2 system. Specific primers were used for the amplification of Macrolide genes by PCR. Among 25 *S. aureus* isolates, 23(92%) isolates were methicillin resistant and 2(8%) isolates were methicilin sensitive. The 5(20%) isolates showed resistance to Erythromycin and sensitivity to Clindamycin with a positive D test which was identified as inducible MLS_B, while 2(8%) isolates showed resistance criteria for both Erythromycin and Clindamycin which identity as a constitutive MLS_B and 18(92%) isolates were given the sensitivity for both Erythromycin and Clindamycin. The *erm*

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resistance genes *(ermA, ermB, ermC, ermF,* and *ermG*) were detected in 5(20%), 17(68%), 25(100%), 24(96%),11(44%) respectively. The D-test, and Vitek ®2 system should be routinely done to avoid treatment failure due to clindamycin resistance.

Keywords---D- test, erm genes, MRSA.

Introduction

Antibiotics of the MLS_B family are frequently used to treat staphylococcal infections. But due to their extensive use, MLS_B antibiotics have seen an increase in the number of staphylococcal strains that are developing resistance to them (Anon *et al.*, 2020). A class of protein synthesis inhibitors with a broad spectrum of activity is known as macrolides (Mokta *et al.*, 2015). The antibiotics known as macrolides included (erythromycin, clarithromycin, azithromycin), lincosamides (clindamycin) and streptogrammins B (quinupristin) (MLS_B) they are associated microbiologically because of their comparable modes of action (Kow and Hasan 2020). Macrolides inhibit protein synthesis by attaching to the bacterial 50S ribosomal subunit's 23S ribosomal RNA, which causes unstable growth of the peptide chain by inhibiting translocation (Bhomi *et al.*, 2016).

Three different mechanisms of resistance of MLS_B antibiotics in *staphylococci*; The first mechanism is modification of the ribosomal target and is encoded by erythromycin ribosome methylase (*erm*) gene which drives to the forming of enzyme methylase (Modukuru *et al.* 2021). The enzyme attaches one or two methyl groups to the adenine residue in 23S rRNA of the 50S ribosomal subunit and preventsthe binding of MLS_B antibiotics to their ribosomal targets (Sarrou *et al.*, 2019; Ferreira *et al.*, 2021). The resistance in the *S. aureus* isolates is due to the MLS_B antibiotics which are possible to be inducible ($iMLS_B$) or constitutive ($cMLS_B$) constitution. In the situation of inducible MLS_B resistance, the bacteria synthesized non-functional mRNA which is not capable of encoding methylase (Heyar *et al.* 2020). Therefore, solely in the presence of a macrolide inducer mRNA could be activated.

On the contrary in $cMLS_B$ resistance, functional methylase mRNA is all the time synthesized even in the lack of an inducer. The strains with $cMLS_B$ are nonsensitive to erythromycin and clindamycin whereas strains with $iMLS_B$ phenotype are resistant to erythromycin and sensitive to clindamycin in-vitro (Moosavian *et al.*, 2014 ; Papkou *et al.*, 2020). The second mechanism of resistance involves an efflux system that encodes the macrolide streptogramin B resistance (*msrA*) gene. The *msrA* gene gives rise to resistance to macrolides and streptogramin B antibiotics (Grgičević *et al.*, 2020). The third mechanism involves enzyme inactivation of antibiotics such as hydrolase, phosphotransferase, nucleotidyltransferase, and lyases (Keenan *et al.*, 2019). Therefore, current researchused the D-test Vitek ®2 system, and PCR to detect the frequency of inducible clindamycin resistance among *S.aureus* isolated isolates from different wounds amples from Al-Basrah governorate, Iraq.

Materials and Methods

Collection of specimens

From October - 2021 to January - 2022, a total of 200 samples in the current study were collected from wound patients that were distributed (50 surgical wounds, 50 burn wounds, 50 gunshot wounds, and 50 broken bones injured) from Al Basrah Teaching and Al Sadder Teaching Hospital in Al-Basrah governorate, Iraq.

Isolation and Identification

The traditional laboratory procedures such as colony morphology, catalase test, slide and tube coagulase testing, and growth on Mannitol Salt agar were used to identify the S. aureus isolated. The confirmed identification was done by the Vitek ®2 system.

Antibiotic susceptibility test

Antibiotic susceptibility testing was performed by the Kirby Bauer disk diffusion method by using cefoxitin (30 µg), oxacillin (1 µg), erythromycin (15 µg), clindamycin (2 µg), Azithromycin (15 µg), and interpreted according to CLSI - (2018) guidelines.

Phenotypic detection of Methicillin resistance

Methicillin resistance was detected by using Cefoxitin (30µg) diskdiffusion (CDD) method, according to CLSI - (2018) guidelines.

Constitutive and inducible clindamycinresistance

Erythromycin (15 µg), and (2 µg), and clindamycin antibiotic disc was used to detect inducible and constitutive resistance to clindamycin according to guidelines of CLSI - (2011, 2012, and 2013), (Nikamet al., 2017 ; Arjyal and Neupane 2020).

Genotypic detection of erm genes
Extraction of Bacterial DNA

Genomic DNA was extracted from the bacterial isolates by using the DNA Presto Mini g DNA Bacteria kit (Geneaid, USA), then DNA bands were detectedby using agarose gel electrophoresis (1%).

Detection of Macrolide and lincosamides Genes

According to Lina et al., (1999), Chen et al., (2007), and Koike et al., (2010). The specific primers pairs were used for amplification of Macrolide genes (ermA, ermB,ermC, ermF, and ermG) genes.

Results

From 200 samples that were collected between October 2021 to January 2022, 58 (29%) samples revealed a positive bacterial growth, whereas 142 (71%) samples revealed a negative bacterial growth. The positive culture was distributed to 25 (43.1%) isolated was *S. aureus* and 33 (56.9%) isolates for different bacterial species include *Psedomonase spp.* 16 (48.5%), *Staphylococcus spp.* 6 (18.18%), *Klebsiella spp.* 5 (15.2%), *E.coli* 4 (12.12%) and *Proteus spp.* 2 (6%) (Fig.1).

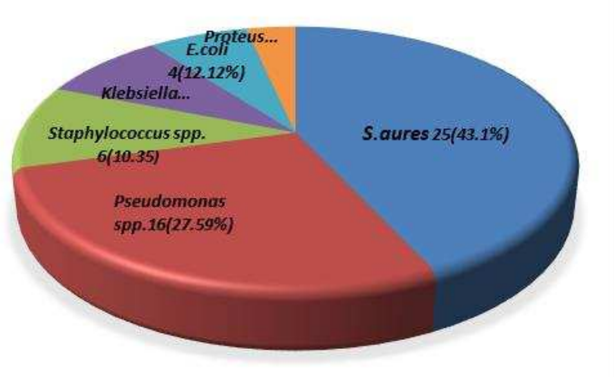

Fig 1. The percentage of the Microorganisms isolates

The results of the antibiotic-resistant test was showing that 23 (92%) *S. aureus* isolates were methicilin-resistant while 2 (8%) were methicillin-sensitive. The 5 (20%) isolates showed resistance to Erythromycin and sensitive to Clindamycin with positive D test which was identyfiy as inducible MLS_B ($iMLS_B$). While the 2(8%) isolates were showed resistance criteria for both Erythromycin and Clindamycin which identify as a constitutive MLS_B ($cMLS_B$). Additionally, the 18 (92%) isolates were given the sensitivity for both Erythromycin and Clindamycin Table (1). The resistance encoding genes *ermA, ermB, ermC, ermA, ermF,* and *ermG* genes results was showed 5 (20%), 17(68%), 25 (100%), 24 (96%), 11 (44%) respectively.

Table 1
D-test result and frequency of cMLSB, IMLSB, and MS phenotypes for *S. aureus* isolates

Susceptibility pattern	Phenotype	Number & Percentage
ERY resistant and CLI sensitive with (D test negative)	MS phenotype	_
(ERY resistant and CLI sensitive with (D test positive)	iMLSB phenotype	5(20%)
ERY resistant and CLI resistant	cMLSB phenotype	2(8%)
ERY sensitive and CLI sensitive	_	18(72%)
Total	25	100%

Discussion

The emergence of drug resistance in methicillin resistance *S. aureus* (MRSA) has resulted in the widespread usage of antibiotics such as macrolide, lincosamide, and streptogramin B (MLS_B). The extensive use of MLS_B antibiotics in severe *staphylococcal* infections has resulted in an outbreak of *S. aureus* resistance to MLS_B antibiotics (Ghanbari *et al.* 2016). The macrolide antibiotics, which act as protein synthesis inhibitors, are often used to treat *staphylococcal* infections. Resistance mechanisms against macrolides include alteration of the ribosomal binding site encoded by the *erm* genes has been found that confers resistance to MLS_B (Nezhad *et al.* 2017 ; Sarrou *et al.* 2019). The study by Bazzi *et al.*, (2017) reported the Vitek ® 2 system was demonstrated to be a method for evaluating the accuracy and speed of direct identification and antibiotic susceptibility testing, also the study by Al-Amara, (2022) was reported Among 28 CoNS isolated, the *S. aureus* 11(39.29%), *Staphylococcus epidermidis*7(25 %), *Staphylococcus haemolyticus* 4(14.29%) and *Staphylococcus saprophyticus* 3 (10.71%) were predominant isolated species. Out of 28 CoNS isolates, 15(53.57%) were methicillin resistant coagulasenegative staphylococci (MRCoNS) isolates and 13(46.43%) were methicillin sensitive coagulase-negativestaphylococci (MSCoNS) isolates. The 15(53.57%) isolates out of 28 CoNS, showed erythromycinresistance while 6(40%) isolates out of 15 CoNS, showed inducible macrolide-lincosamide-streptograminB (iMLSB) and 2(13.3%) of CONS isolated showed constitutive macrolide-lincosamide-streptogramin B(cMLSB)

Clindamycin is an excellent and preferred agent to treat superficial infections with *S. aureus* and a preferred antibiotic in patients allergic to penicillin. Resistance to clindamycin in *S. aureus* strains with inducible phenotype may be reported as sensitive if not tested by D-test giving a false sensitive report which could result in treatment failure and also the emergence of constitutive *erm* mutants (Modukuru *et al.* 2021). The CLSI proposed in 2013 that the D-zone test, a phenotypic approach, be used to screen for inducible clindamycin resistance. All erythromycin-resistant *S.aureus* is also recommended for testing of inducible clindamycin resistance to clindamycin treatment failures and reporting of prevalence-resistant phenotypes (Jha *et al.* 2019).

In the present study, the result of MLS_B was shown the prevalence of $iMLS_B$ followed by $cMLS_B$ and not detected any isolated handling of the MS resistance when tested by using the phenotypic method. PCR investigation for detecting the macrolide antibiotics resistant genes was shown the *ermC* genes as dominant in all isolates, followed by *ermF, msrA, msrB*, *ermB,* and *ermG* in 25 (100%), 24 (96%), 17 (68%),11 (44%) and, 5 (20%) respectively. The variable presence of erythromycin resistance may explain differences in the prevalence rate of different investigations of MLS_B resistance genes (Khoshnood *et al.* 2019)

The D-zone test results in the study of Fasihi *et al.*, (2017) revealed the inducible clindamycin resistance in 12.5% (21/170) and *S. aureus*were harboring *mecA, erm(A), erm(B),and erm(C)* {39.5% (69/170), 11% (19/170), 3.5% (6/170), and 20.5% (35/170)} respectively. The study by Ghanbari *et al.*; (2016) reported the frequency of $cMLS_B$, and $iMLS_B$ phenotypes as 58 (26.9%), and 9 (4.18%) respectively. Furthermore, the frequency of *ermC, ermB*, and *ermA* genes among

S. aureus isolates with iMLSB was 44.4%, 22.2%, and 11.1% respectively. In a study by Khashei *et al.*; (2018) it had been detected that the prevalence of cMLSB and iMLSB phenotypes in *S. aureus* isolated from various clinical samples was 29 (82.9%) respectively. Also identified were the predominant *ermC* genes in 29 (82.9%), and *ermA* genes in 20 (57.1%). In Iran the study of Khoshnood *et al.*, (2019) revealed that the MRSA isolates were examined for the presence of *ermB, ermA,and ermC* genes as the primary cause of macrolide resistance. The occurrence rates of*, ermA*, and *ermC* genes in MRSA isolates were 28 (46.7%), and 22 (36.7%), respectively. Also, The study by Cevahir and Kaleli, (2015) found that among 120 *S. aureus* isolates, 85 (70.8%) were methicillin-resistant *S. aureus* (MRSA), and 35 (29.2%) were methicilin-sensitive *S. aureus* (MSSA). The tested isolates contained resistance genes, including *ermA* (26.7%), *ermB* (10.8%), *ermC* (11.7%).

The study by Goudarzi, Eslami, *et al.*, (2019) found among 120 *S. aureus* isolates, 85 (70.8%) were methicillin-resistant *S. aureus* (MRSA), and 35 (29.2%) were methicilin sensitive S. aureus (MSSA). The tested isolates contained resistance genes, including *ermA* (26.7%), *ermB* (10.8%), *ermC* (11.7%). Also, The study of Elsayed *et al.*, (2019) demonstrated the high antimicrobial resistance of the investigated isolates. A total of 20 methicillin-resistant *S. aureus* (MRSA) isolates. The 12 MRSA isolates harbored the methicillin resistance genes *mecA* 9/12 (75%). The distributions of *erm(A), erm(B), erm(C), erm(F),* and *erm(G)* were 8/12 (66.7%), 5/12 (41.7%), 12/12 (100%), 2/12 (16.7%), and 0/12 (0.0%) respectively.

Conclusions

The D-test, and Vitek ®2 system should be routinely done to avoid treatment failure due to clindamycin resistance. In addition, the PCR technique should be performed for the detection of genes responsible for erythromycin resistance as it is a quick and most sensitive method.

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References

1. AlAl-Amara,S.,S.,M.(2022).,Constitutive and Inducible ClindamycinResistance Frequencies amongStaphylococcus sp. Coagulase Negative Isolatesin Al-Basrah Governorate, Iraq ,Rep. Biochem. Mol. Biol, 11(1):31-35
2. Anon, Sneh Lata, Rosy Bala, Neerja Jindal, and Nitin Gupta. 2020. “In Vitro Study of Constitutive and Inducible Clindamycin Resistance in Staphylococcus Aureus with Reference to Methicillin Resistant Staphylococcus Aureus: Experience From Tertiary Care Hospital in Punjab.” Indian Journal of Public Health Research & Development 11(2): 314.

3. Arjyal, Charu, Jyoti Kc, and Shreya Neupane. 2020. "Prevalence of Methicillin-Resistant Staphylococcus Aureus in Shrines." International Journal of Microbiology 2020.
4. Bazzi, Ali M, Ali A Rabaan, Mahmoud M Fawarah, and Jaffar A Al-Tawfiq. 2017. "Direct Identification and Susceptibility Testing of Positive Blood Cultures Using High Speed Cold Centrifugation and Vitek II System." Journal of Infection and Public Health 10(3): 299–307.
5. Bhomi, Ujwol et al. 2016. "Status of Inducible Clindamycin Resistance among Macrolide Resistant Staphylococcus Aureus." African Journal of Microbiology Research 10(9): 280–84.
6. Chen, Jing et al. 2007. "Development and Application of Real-Time PCR Assays for Quantification of Erm Genes Conferring Resistance to Macrolides-Lincosamides-Streptogramin B in Livestock Manure and Manure Management Systems." Applied and Environmental Microbiology 73(14): 4407–16.
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty - Second Informational Supplement. CLSI document M100 -S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012 .
8. Djunaid, R., Sahar, J., Widyatuti, W., & Priyo, S. (2022). Predictive factors contributing to violent behavior in adolescents . International Journal of Health Sciences, 6(2), 1148–1159. https://doi.org/10.53730/ijhs.v6n2.11483
9. Elsayed, Mohamed Sabry Abd Elraheam et al. 2019. "Phenotypic and Genotypic Methods for Identification of Slime Layer Production, Efflux Pump Activity, and Antimicrobial Resistance Genes as Potential Causes of the Antimicrobial Resistance of Some Mastitis Pathogens from Farms in Menoufia, Egypt." Molecular Biology Reports 46(6): 6533–46. https://doi.org/10.1007/s11033-019-05099-6.
10. Fasihi, Yasser, Fereshteh Saffari, Mohammad Reza Kandehkar Ghahraman, and Davood Kalantar-Neyestanaki. 2017. "Molecular Detection of Macrolide and Lincosamide-Resistance Genes in Clinical Methicillin-Resistant Staphylococcus Aureus Isolates from Kerman, Iran." Archives of Pediatric Infectious Diseases 5(1): 4–8.
11. Ferreira, Carolina et al. 2021. "Clonal Lineages, Antimicrobial Resistance, and Pvl Carriage of Staphylococcus Aureus Associated to Skin and Soft-Tissue Infections from Ambulatory Patients in Portugal." Antibiotics 10(4).
12. Ghanbari, Fahimeh et al. 2016. "Distribution of Erm Genes among Staphylococcus Aureus Isolates with Inducible Resistance to Clindamycin in Isfahan, Iran." Advanced Biomedical Research 5(1): 62.
13. Goudarzi, Mehdi et al. 2019. "Clonal Dissemination of Staphylococcus Aureus Isolates Causing Nosocomial Infections, Tehran, Iran." Iranian Journal of Basic Medical Sciences 22(3): 238–45.
14. Grgičević, Ivan et al. 2020. "Discovery of Macrozones, New Antimicrobial Thiosemicarbazone-Based Azithromycin Conjugates: Design, Synthesis and in Vitro Biological Evaluation." International Journal of Antimicrobial Agents 56(5).
15. Heyar, Avneet Kaur, Kamaldeep Kaur, Amarjit Kaur Gill, and Prabhjot Kaur Gill. 2020a. "Induction of Clindamycin Resistance in Clinical Isolates of Staphylococcus Aureus From a Tertiary Care Hospital." International Journal of Medical and Biomedical Studies 4(12).

16. Institute, CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty - Third Informational Supplement. 28th edn. Edited by P. Wayne. Wanye, PA Clinical and Laboratory Standards Institute Antimicrobial, 2018.
17. Jha, Awadhesh Kumar et al. 2019. “Determine Constitutive and Inducible Clindamycin Resistance among Clinical Isolates of Staphylococcus Aureus Isolates from Tertiary Care Hospital, Bettiah, India.” International Journal of Contemporary Medical Research [IJCMR] 6(11): 1–5.
18. Keenan, Jeremy D. et al. 2019. “Longer-Term Assessment of Azithromycin for Reducing Childhood Mortality in Africa.” New England Journal of Medicine 380(23): 2207–14.
19. Khashei, Reza, Yalda Malekzadegan, Hadi Sedigh Ebrahim-Saraie, and Zahra Razavi. 2018. “Phenotypic and Genotypic Characterization of Macrolide, Lincosamide and Streptogramin B Resistance among Clinical Isolates of Staphylococci in Southwest of Iran.” BMC Research Notes 11(1): 1–6.
20. Khoshnood, Saeed et al. 2019a. “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): 387–98.
21. Koike, Satoshi et al. 2010. “Molecular Ecology of Macrolide-Lincosamide-Streptogramin B Methylases in Waste Lagoons and Subsurface Waters Associated with Swine Production.” Microbial Ecology 59(3): 487–98.
22. Kow, Chia Siang, and Syed Shahzad Hasan. 2020. “Use of Azithromycin in COVID 19: A Cautionary Tale.” Clinical Drug Investigation 40(10): 989–90. https://doi.org/10.1007/s40261-020-00961-z.
23. Lina, Gerard et al. 1999a. “Distribution of Genes Encoding Resistance to Macrolides, Lincosamides, and Streptogramins among Staphylococci.” Antimicrobial Agents and Chemotherapy 43(5): 1062–66.
24. Modukuru, Giridhar Kumar, Pradeep Madala Sobhana Surya, Vishnuvardhana Rao Kakumanu, and Saritha Yarava. 2021. “Phenotypic Characterization of Macrolide-Lincosamide-Streptogramin B Resistance in Staphylococcus Aureus.” Journal of Pure and Applied Microbiology 15(2): 689–94.
25. Mokta, Kiran K. et al. 2015. “Inducible Clindamycin Resistance among Clinical Isolates of Staphylococcus Aureus from Sub Himalayan Region of India.” Journal of Clinical and Diagnostic Research 9(8): DC20–23.
26. Moosavian, Mojtaba et al. 2014. “Inducible Clindamycin Resistance in Clinical Isolates of Staphylococcus Aureus Due to Erm Genes, Iran.” Iranian Journal of Microbiology 6(6): 421–27.
27. Nezhad, Ramin Rashidi et al. 2017. “Molecular Characterization and Resistance Profile of Methicillin Resistant Staphylococcus Aureus Strains Isolated from Hospitalized Patients in Intensive Care Unit, Tehran-Iran.” Jundishapur Journal of Microbiology 10(3).
28. Nikam, Archana P., Pramod R. Bhise, and Mukta M. Deshmukh. 2017. “Phenotypic Detection of Inducible Clindamycin Resistance among Staphylococcus Aureus Isolates.” International Journal of Research in Medical Sciences 5(2): 543.
29. Papkou, Andrei et al. 2020. “Efflux Pump Activity Potentiates the Evolution of Antibiotic Resistance across S. Aureus Isolates.” Nature Communications 11(1). http://dx.doi.org/10.1038/s41467-020-17735-y.

30. Sarrou, Stela et al. 2019. "MLSB-Resistant Staphylococcus Aureus in Central Greece: Rate of Resistance and Molecular Characterization." Microbial Drug Resistance 25(4): 543–50.
31. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. International Journal of Health Sciences, 6(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
32. Widana, I. K., Sumetri, N. W., & Sutapa, I. K. (2018). Effect of improvement on work attitudes and work environment on decreasing occupational pain. International Journal of Life Sciences, 2(3), 86–97. https://doi.org/10.29332/ijls.v2n3.209