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RESEARCH ARTICLE - MEDICAL TECHNIQUES

Phenotypic Resistance of (MRSA) Clinical Isolates to Some Macrolide Antibiotic Groups

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Abstract

Antimicrobial resistance is one of the most significant global threats to human health in recent times, and it limits the achievement of several of the sustainable development goals. Methicillin-resistant Staphylococcus aureus (MRSA) is among the most essential multidrug-resistant bacterial pathogens. The study aimed to determine the prevalence of (MRSA) from different clinical samples to emphasise the suitable treatment. One hundred fifty different clinical samples were collected. All these samples were subjected to classical microbiological testing, which included culturing directly on mannitol salt agar and antibiotic susceptibility test (A.S.T). The results observed that only 32 out of 150 samples of MRSA isolates were identified using cefoxitin 30 (µg) as a screening antibiotic, as suggested by CLSI. These 32 MRSA isolates showed resistance toward penicillin and cefoxitin, high sensitivity against vancomycin, and moderate resistance against doxycycline and azithromycin. Considering the phenotyping resistance toward macrolide antibiotic groups, 10/32 (31.25%) showed S-phenotype,18/32 (52.65%) showed R-phenotype,4/32 (12.5%) showed D-phenotype while no MRSA isolated showed D+ phenotype. This study concludes that inducible clindamycin resistance of S. aureus (MRSA) increases the difficulty of treating S. aureus bacterial infections.

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Keywords: Burn Samples; Cefoxitin; D-Phenotype; Erythromycin; MRSA.

1. Introduction

The World Health Organisation (WHO) warned about the potential end of the era of effective antibiotics in 2014 [1]. *Staphylococcus aureus* was included on the World Health Organization's (WHO) list of 12 pathogens that pose hazards to public health and for which the identification of effective medicines is a priority three years later [2,3]. The family Staphylococcaceae consists of 40 different species of bacteria, both harmful and harmless. Because of its multiple virulence characteristics, *S.aureus* is a primary causative agent of various illnesses. Infections caused by *S. aureus* range from those affecting the skin and soft tissues to those affecting the urinary tract, the respiratory system, and even the skeletal system. Sepsis, septic shock, and opportunistic infections are all possible outcomes of a staphylococcal infection [4-6]. To effectively combat staphylococcal drug resistance in the future, it is crucial to comprehend the mechanisms of staphylococcal resistance [2]. In 1928, Alexander Fleming found that mould might prevent staphylococci from multiplying. In this case, penicillin was the key element [6]. When Fleming received the Nobel Prize for his discovery, he expressed concern that penicillin resistance might emerge. Only 20 years after penicillin's discovery, the globe was ruled by staphylococcal strains resistant to this antibiotic, just as he had prophesied. Semi-synthetic penicillins (such as methicillin) were introduced to treatment in 1959 as a reaction to the rising penicillin resistance. However, barely a year in the future, the first methicillin-resistant *S.aureus* (MRSA) strain was found, and by the end of the next decade, MRSA was the leading cause of outbreaks in hospitals worldwide. Several-resistant*Staph. aureus* (MRSA) isolates are created by the horizontal gene transfer of several clones of the SCCmec chromosomal cassettes into *S. aureus* [7]. Molecular biology methods from the 1990s showed a clonal spread of *S. aureus* in a hospital setting. Overuse of antibiotics effective against Gram-positive bacteria has led to the emergence of staphylococcal isolates resistant to the commonly used macrolide, lincosamide, and streptogramin B classes of drugs. The erm and msr genes control resistance to MLSB antibiotics. Due to its quick acquisition of resistance and the scarcity of treatment options available, methicillin-resistant *Staphylococcus aureus* (MAC-MRSA) has emerged as one of today's most therapeutically significant bacteria [8]. While only around 40% of methicillin-sensitive *S. aureus* (MSSA) strains are resistant to MLSB drugs, over 80% of MRSA strains are. Several mechanisms contribute to *S.aureus's* ability to resist methicillin and macrolides, including altering the antibiotic's target site (through erm genes) and actively excreting macrolides within the bacterial cell (by M.S.R. genes). Because of these processes, treatment options for MRSA infections are severely constrained [9,11]. Controlling the extension of antibiotic resistance is essential because of the high incidence of MLSB resistance in MRSA isolates. Additionally, since *S. aureus* has developed resistance to several life-saving antibiotics like vancomycin, the idea that older, less frequently used antibiotics like macrolides remain effective in relating to staphylococcal infections appears promising in preventing the rise of new resistances [4]. The effectiveness of macrolide antibiotics spans a wide range of bacteria.

Nomenclature & Symbols			
C F U	Colony Forming Unit	MRSA	Methicillin Resistance S.Aueurs
CLSI	Clinical and Laboratory Standards Institutes	MSSA	Methicillin-Sensitive *S.Aureus*
F.E.P	Fisher's Exact Probability Test	NCTL	National Center for Teaching Laboratory
HA-MRSA	Health-Care-Associated MRSA	WHO	World Health Organization
MSA	Mannitol Salt Agar	IZDs	Inhibition Zoned Diametres
D d	Shape Letter		

They have a broad spectrum of antibacterial activity, including against Gram-positive also Gram-negative bacteria. Although the spectrum of actions for various macrolides may vary significantly, this is typically the case. These variations emerge because the pharmacokinetic parameters are chemically structure-dependent [8]. Macrolides are the mainstay treatment for MSSA infections nowadays. Infections of the upper respiratory tract, community-acquired pneumonia, the skin, the gut, and infections caused by Salmonella and Shigella are all treated with these drugs. The macrolides have excellent cellular penetration, particularly into granulocytes and macrophages. Additionally, they accumulate to substantial levels in cells as well as tissues. Since macrolides contribute to intracellular killing mechanisms, they are effective against pathogens that can live only inside cells, including *S. aureus* [8]. In light of this, this research aims to investigate the MRSA isolates' phenotypic susceptibility profile to a selection of macrolide antibiotics.

2. Materials and Methods

2.1. Specimen collection

One hundred and fifty clinical samples were isolated from different clinical sources of infections (Burn, diabetic ulcer, urine, sputum, wound swabs, ear swabs) from the National Center for Teaching Laboratory (NCTL), Baghdad Teaching Hospital, Gazzi Al-Harrii Hospital, and AL-Zafranya hospital.

2.1.1. Isolation and identification of S.aueurs bacteria

Samples were streaked on Blood-agar and Mannitol salt agar (M.S.A.). After overnight incubation, all the suspected colonies as *S.aueurs* were subjected to further phenotypic tests, including colony morphology detection, gram stain method, catalase test, and coagulase test.

2.1.2. Methicillin resistance S.aueurs (MRSA) screening

Following protocols, all strains were tested for methicillin resistance with oxacillin (1µg) and cefoxitin (30 µg) disc diffusion [12, 13].

2.1.3. The testing of antibiotic susceptibility

The disc diffusion (Kirby-Bauer) method was used to analyse MRSA isolates susceptibility profiles. McFarland 0.5 values were prepared of bacteria and then inoculated onto Mueller-Hinton agar plates. The tested antibiotics included oxacillin (1µg) and cefoxitin (30µg), vancomycin (30µg), linezolid, clindamycin (2µg), erythromycin (15µg), azithromycin (15µg), doxycycline (30 µg) were distributed on the media. After 24 hrs incubation a 35°C, zone diameters were measured according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2022). The zone inhibition diameters were explained.

2.1.4. Disk approximation test with erythromycin and clindamycin (D-Zone test)

The disc approximation test with erythromycin & clindamycin (D-zone test) was used to detect inducible clindamycin resistance, as suggested by CLSI (CLSI, 2022). Overnight-grown organisms were used to make 0.5 McFarland suspensions, which were then used to inoculate and distribute throughout the surface of Mueller-Hinton's agar plates. Discs of erythromycin (15 µg) and clindamycin (2µg) (Bioanalyzed, Turkey) were spaced 15 mm apart on the inoculation plates. After incubating the plates for 18 hours at 35°C, the inhibition zoned diametres (I.Z.D.s) was measured in the case of a flattening shape of the clindamycin inhibitory zone (D shape) around the erythromycin disk, which explained that erythromycin had caused clindamycin resistance. The staphylococcal isolates can also be classified into several phenotypes. These include the S phenotype, which (is sensitive to both erythromycin and Clindamycin); the R phenotype had constitutive resistance and was (resistant to both erythromycin and clindamycin); the D phenotype was (resistant to both erythromycin and clindamycin) in a zone like D; and the D+ phenotype was (resistant to both erythromycin and clindamycin) in a zone like D with small colonies growing within the D zone [14].

2.2. Statistical analysis

Statistical Analysis System version (2021) program was utilized to determine the impact of various factors on the study's parameters. The Chi-square test and Fisher exact probability (F.E.P) were used in this study to compare percentages significantly, and 0.05 was the level of significance [15].

3. Results

Thirty-two MRSA isolates could be picked up from different sample types: 8/32 (25.0%) and 8 /32 (25.0%) isolated from burn and wound swabs, respectively sample. In contrast, the lower cases of MRSA were isolated from sputum samples with similar cases 2/32 (12.50%), 1 (50.0%), Ear swab and diabetic foot ulcer samples had equal and percentages of MRSA isolates 4/32 (21.50%), 4/32 (21.50%) respectively as arranged in Table 1.

Thirty- two MRSA infections could pick up from different samples type; there were equal cases 4 (66.7%, 80.0%,57.1%) cases recorded among male groups at the age groups (31-40),(41-50),(51-60) years, respectively versus equal cases of MRSA infection 2 (50.0%,40.0%,33.0%) were recorded at female age group (50.0%,40.0%,33.0%) respectively, these differences statistically were nonsignificant (F.E.P=1.95, p-value=0.85) as arranged in Table 2.

Table 1. Frequency and percentage of MRSA isolates isolated from various clinical samples

Clinical samples	Total
Burn	8 (25.0%)
Wound swabs	8(18.75%)
Urine	6 (25.0%)
diabetic ulcer	4 (12.5.0%)
Ear swabs	4 (21.50%)
Sputum	2 (6.25.0%)
Total	32 (100.0%)

Table 2. Frequency and percentage of MRSA isolates isolated from various age groups (Years) distributed according to Sex

Age range (Years)	Sex			P-value
	Male	Female		
(11-20)	2 (50.0%)	2 (50.0%)	4 (100.0%)	
(21-30)	3 (60.0%)	2 (40.0%)	5 (100.0%)	F.E.P=1.95
(31-40)	4 (66.7%)	2 (33.3%)	6 (100.0%)	P-value=0.85
(41-50)	4 (80.0%)	1 (20.0%)	5 (100.0%)	(N.S)
(51-60)	4 (57.1%)	3 (42.9%)	7 (100.0%)	
>60	2 (40.0%)	3 (60.0%)	5 (100.0%)	
Total	19 (59.4%)	13 (40.6%)	32 (100.0%)	

The results of the antibiotic susceptibility test documented that all the *S.auerus* isolates were resistant to (penicillin 100,0%) and cefoxitin (100.0%). In comparison, the sensitivity towards doxycycline antibiotic were (81.3%), and the majority of *S.aueurs* isolates were resistant mostly against azithromycin (87.5%). A lower resistance rate was recorded against clindamycin (62.5%), as shown in Table 3 and Fig. 1.

The current study showed four phenotypic patterns in MRSA isolates (Fig. 1): ten (31.25%) observed sensitivity to E and CD antibiotics. Eighteen (56.25%) were resistant to both E and CD antibiotics. Four (12.5%) showed resistance to both erythromycin and Clindamycin and resembled a D-shaped zone, which indicates inducible resistance to clindamycin, as explained in Figs. 2 and 3.

Table 3. The results of the antibiotic susceptibility profile of MRSA isolates (n=32)

Antibiotic	Abbreviation	Conc.	Sensitive (%)	Intermediate (%)	Resistance (%)
Pencilline	P	10 (U)	-	-	32 (100%)
Cefoxitin	C.F.X.	30 (µg)	-	-	32 (100%)
Doxycycline	DO	30 (µg)	26 (81.3%)	5 (15.6%)	1 (3.1%)
Azithromycin	AZM	15 (µg)	4 (12.5%)	-	28 (87.5%)
Vancomycin	VAN	30 (µg)	32 (100%)	-	-

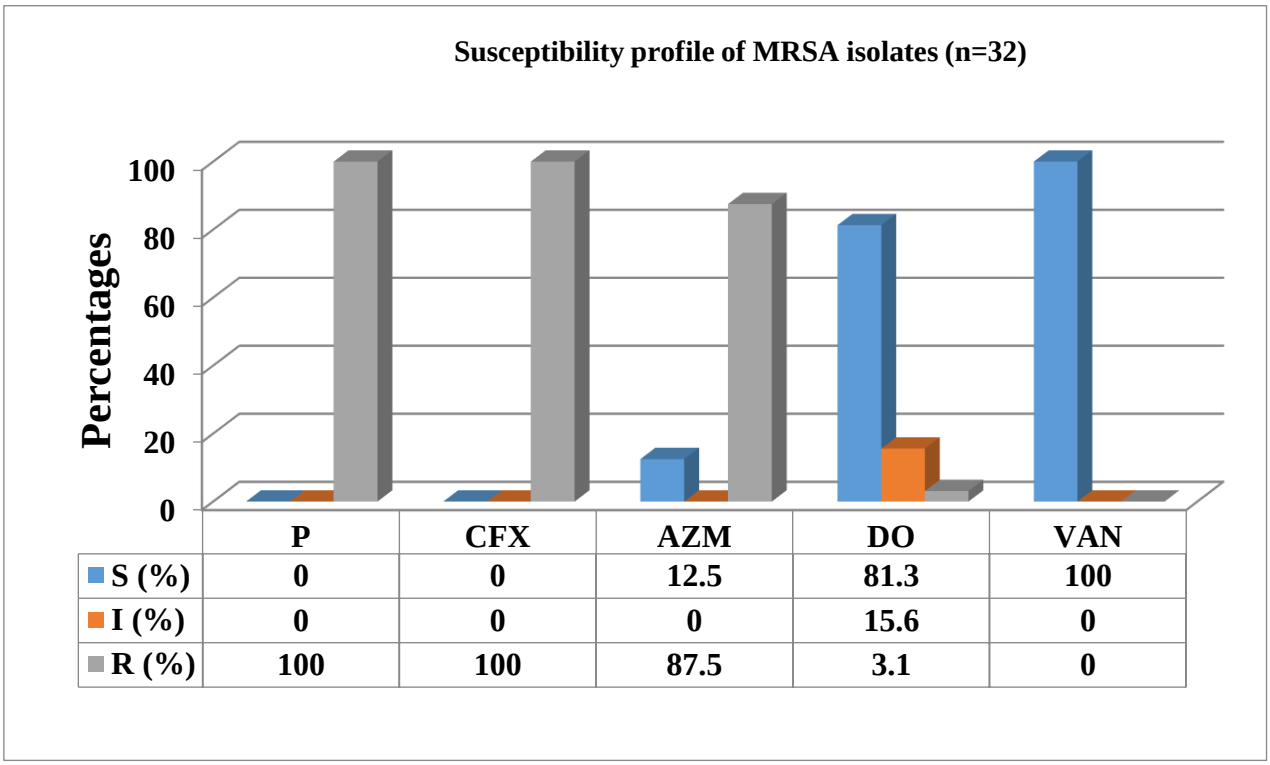

Fig. 1. The results of antibiotic susceptibility profile of MRSA isolates (n=32)

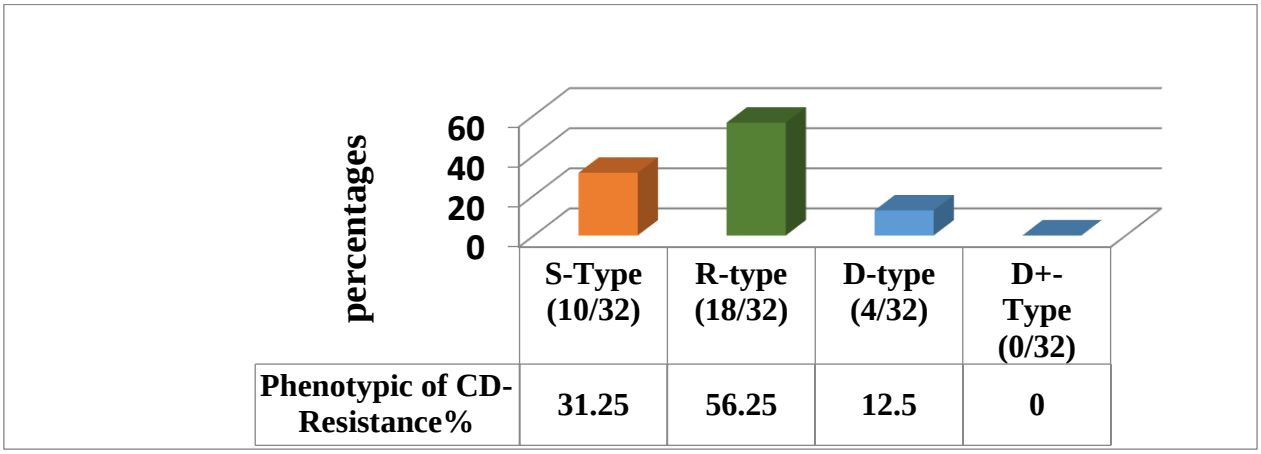

Fig. 2. Phenotypic representation of clindamycin resistance among MRSA

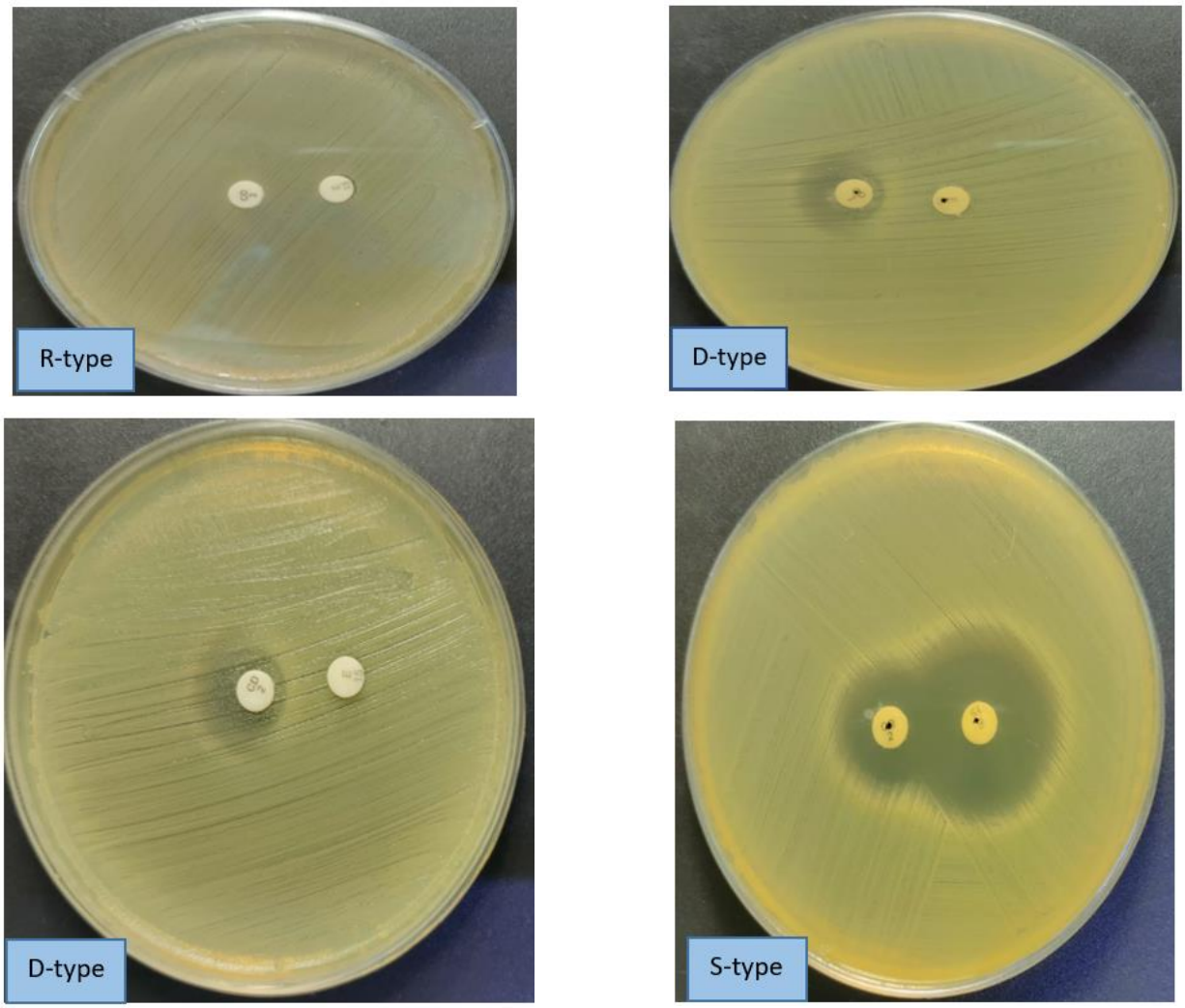

Fig. 3. Phenotypic representation of clindamycin resistance showing MRSA in M.H.A. media

4. Discussion

Antibiotics have wholly changed how bacterial illnesses are treated. Unfortunately, the selection and development of antibiotic-resistant bacteria and a higher treatment failure ratio have resulted from the extensive and improper use of antibacterial medications in both human and veterinary health and the food sector. [2] *Staphylococcus aureus* was included on a list of 12 pathogens released by the World Health Organisation (WHO) that pose hazards to public health and for which the identification of effective medicines is a priority [2,3]. The family Staphylococcaceae consists of 40 different species of bacteria, both harmful and harmless. Because of its multiple virulence characteristics, *S. aureus* is a primary causative agent of a wide variety of illnesses. Infections caused by *S. aureus* can affect any part of the body, from the skin and soft tissues to the urinary tract, the lungs, and the bones. Sepsis, septic shock, and opportunistic infections are all possible outcomes of staphylococcal infection

[4,5].Understanding the causes of staphylococcal resistance is crucial for future success in the battle against drug-resistant bacteria [2]. Due to the breakdown of the skin's protective barrier, microbes can swiftly colonize and multiply following a burn [16]. The findings of this study revealed that 8/32 (25.0%) and 8/32 (25.0%) of MRSA infections were identified from burn and wound swab samples. Gram-positive bacteria such as Staphylococcus species, Enterococcus species, and beta-hemolytic Streptococcus of group A are frequently involved in burn wound infections. *S. aureus* is one of the leading bacterial causes of infections in burn wounds [17]. Conclusions from this study were harmonized with the results of [18], who documented that *S. aureus* was the second most prevalent bacteria found in contaminated burn wounds, accounting for 24.05% of the total samples. Our results were also in agreement with the results of [19], who documented that *S.auerus* is the most common pathogen detected in burn and injury patients 30/57(52.6%) and 27(47.4%) isolated from burns and wounds, respectively. Inflammatory skin surface microbes are classified as pyogenic bacteria, according to research by Ekawati et al. One of the pyogenic bacteria is *S. aureus* [20]. Also, Gilmara *et al*. and colleagues [21] found that nasal and skin colonization by Staphylococcus sp. may be a source for *S. aureus* colonization of wounds and that *S. aureus* and MRSA colonize a sizable percentage of wounds. Another form of a wound is a burn; William *et al*. and coworkers observed that MRSA, often spread in hospitals, is increasingly becoming the causative agent of invasive infection in burn patients [22]. The frequency of MRSA infection was higher among males 19/32 (59.4%) than females 13/32 (40.6%). Regarding age groups, the frequency of MRSA among male groups was primarily recorded in the third to sixth decade of ages, and These results were in agreement with the results of [23], the study which documented that most groups have been infected with *S. aureus* in male was at (70-79) years. The results of the test showed that the responses of the different bacterial isolates to the medicines were somewhat variable. This result was comparable to that of a study [24], in which it was found that the isolates obtained from the investigation of the antibiotics used showed a resistance rate of one hundred percent to the antibiotic penicillin at the highest rates. Bacteria were resistant to penicillin by 100, more significant than the 93.5 percent seen in earlier research [25]. This could be because the stimulating bacteria become more resistant to it over time as a result of its irregular and ongoing usage.

Conclusions from the current study found that the isolates were resistant to doxycycline at a rate of (3.1%), which is not comparable to the findings of [26,27], who recorded a resistance rate of doxycycline of 20.6% and 30%, respectively. azithromycin had a resistance rate of 28 (87.5%) of MRSA isolates, which was less low than the results of [28], who published that the resistance rate of MRSA isolates against azithromycin was 92.5%. In comparison, this study's resistance rate of cefoxitin was 100%, which is higher than the results of [28], which was 97.5%. The resistance rate of all the MRSA isolates of this study was not observed resistance to vancomycin with 100% sensitivity, and these results were approached with results of [29,30], who documented the MRSA isolates were high sensitivity to vancomycin at 4%,10% of the resistance. Increased resistance to Macrolide-Lincosamide-Streptogramin type B antibiotics has been linked to the rise of methicillin-resistant *Staphylococcus aureus* in both hospital- and community-acquired infections. As a result, therapy has become more challenging, which has increased morbidity, hospitalization duration, and overall treatment costs. As a result, it is vital that isolates of *S. aureus* and their sensitivity patterns, particularly towards methicillin, clindamycin, and erythromycin, are correctly identified and reported [13]. Based on our findings, inducible resistance to clindamycin was present in 12.5% of all isolates tested. Our results were not harmonized with the results of [29], which documented that the percentage of inducible clindamycin resistance among MRSA isolates was 13 (76.4%). The results of this study showed, which depended on the phenotypic D-test that the sensitive, constitutive, and inducible resistance with D zone phenotypes isolates was 31.25%,56.25%,12.5%, which semi-harmonized with results of [13] who showed that the 32.3% of MRSA isolates revealed inducible resistance with D zone, whereas 54.8 percent of isolates and 12.9 percent of isolates were found to have the sensitive and constitutive phenotypes, respectively.

5. Conclusions

This study concludes that *S. aureus* (MRSA), with high antibiotic resistance, was the most prevalent from wound swabs and burn samples. The results also observed that the MRSA isolates with sensitive and constitutive clindamycin resistance are more in number than the inducible phenotype. The identification of inducible resistance in isolates of Staphylococcus aureus can be accomplished by utilizing the D-test. This test can be implemented as a standard procedure in all microbiology laboratories, thereby aiding healthcare professionals in preventing treatment failure associated with Clindamycin.

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Ethical Approval

The Medical City Ethical Commission in the Iraqi Ministry of Health certified this research. Health Administration (804 at 2023.1.8).

References

[1] S. Reardon, "WHO warns against ‘post-antibiotic era," Nature, vol. 15, pp. 135-8, 2014. https://doi.org/10.1038/nature.2014.15135.
[2] R. E. Duval, M. Grare, and B. Demoré, "Fight against antimicrobial resistance: we always need new antibacterials but for right bacteria," Molecules, vol. 24, no. 17, p. 3152, 2019. doi: 10.3390/molecules24173152.
[3] M. Mišić et al., "Prevalence of genotypes that determine the resistance of staphylococci to macrolides and lincosamides in Serbia," Frontiers in Public Health, vol. 5, p. 200, 2017. doi: 10.3389/fpubh.2017.00200.
[4] D. Oliveira, A. Borges, and M. Simões, "Staphylococcus aureus toxins and their molecular activity in infectious diseases," Toxins, vol. 10, no. 6, p. 252, 2018. DOI: 10.3390/toxins10060252.
[5] K. Kang and S. Redner, "Fluctuation-dominated kinetics in diffusion-controlled reactions," Physical Review A, vol. 32, no. 1, p. 435, 1985. DOI: 10.1103/physreva.32.435.
[6] S. De Backer et al., "Enzymes catalysing the TCA-and urea cycle influence the matrix composition of biofilms formed by methicillin-

resistant Staphylococcus aureus USA300," Microorganisms, vol. 6, no. 4, p. 113, 2018. DOI: 10.3390/microorganisms6040113.

[7] A. S. Bishr, S. M. Abdelaziz, I. S. Yahia, M. A. Yassien, N. A. Hassouna, and K. M. Aboshanab, "Association of Macrolide Resistance Genotypes and Synergistic Antibiotic Combinations for Combating Macrolide-Resistant MRSA Recovered from Hospitalized Patients," Biology, vol. 10, no. 7, p. 624, 2021. doi: 10.3390/biology10070624.

[8] G. Mancuso, A. Midiri, E. Gerace, and C. Biondo, "Bacterial antibiotic resistance: The most critical pathogens," Pathogens, vol. 10, no. 10, p. 1310, 2021. DOI 10.3390/pathogens10101310.

[9] G. P. Dinos, "The macrolide antibiotic renaissance," British Journal of Pharmacology, vol. 174, no. 18, pp. 2967-2983, 2017. DOI: 10.1111/bph.13936.

[10] W. Yao et al., "Staphylococcus aureus with an erm-mediated constitutive macrolide-lincosamide-streptogramin B resistance phenotype has reduced susceptibility to the new ketolide, solithromycin," BMC infectious diseases, vol. 19, pp. 1-8, 2019. DOI:10.1186/s12879-019-3779-8.

[11] M. Miklasińska-Majdanik, "Mechanisms of resistance to macrolide antibiotics among Staphylococcus aureus," Antibiotics, vol. 10, no. 11, p. 1406, 2021. doi.org/10.3390/antibiotics10111406.

[12] S. Armin, F. Fallah, M. Navidinia, and S. Vosoghian, "Prevalence of blaOXA-1 and blaDHA-1 AmpC β-lactamase-producing and methicillin-resistant Staphylococcus aureus in Iran," Archives of Pediatric Infectious Diseases, vol. 5, no. 4, 2017. DOI: https://doi.org/10.5812/pedinfect.36778.

[13] E. P. L. Pereira-Franchi, M. R. N. Barreira, N. d. S. L. M. d. Costa, C. M. C. B. Fortaleza, and M. d. L. R. d. S. d. Cunha, "Prevalence of and risk factors associated with the presence of Staphylococcus aureus in the chronic wounds of patients treated in primary health care settings in Brazil," Revista da Sociedade Brasileira de Medicina Tropical, vol. 50, pp. 833-838, 2017. 10.1016/j.bjid.2021.101636.

[14] D. George and P. Mallery, "IBM SPSS Statistics 26 Step by Step A Simple Guide and Reference sixteenth edition," ed: Taylor & Francis, 2020.

[15] C. G. Mayhall, Hospital epidemiology and infection control. Lippincott Williams & Wilkins, 2012.

[16] C. E. Ramirez-Blanco, C. E. Ramirez-Rivero, L. A. Diaz-Martinez, and L. M. Sosa-Avila, "Infection in burn patients in a referral centre in Colombia," Burns, vol. 43, no. 3, pp. 642-653, 2017. DOI: 10.1016/j.burns.2016.07.008.

[17] N. A. Chaudhary et al., "Epidemiology, bacteriological profile, and antibiotic sensitivity pattern of burn wounds in the burn unit of a tertiary care hospital," Cureus, vol. 11, no. 6, 2019. doi: 10.7759/cureus.4794.

[18] M. K. Khudhair and M. A. AlAubydi, "Determination the prevalence and antimicrobial susceptibility of bacteria isolated from burns and wounds," Iraqi Journal of Agricultural Sciences, vol. 54, no. 1, pp. 93-99, 2023. DOI: https://doi.org/10.36103/ijas.v54i1.1679.

[19] E. Ekawati, W. Darmanto, and S. Wahyuningsih, "Detection of Staphylococcus aureus in wound infection on the skin surface," in IOP Conference Series: Earth and Environmental Science, 2020, vol. 456, no. 1: IOP Publishing, p. 012038. DOI 10.1088/1755-1315/456/1/012038.

[20] G. C. M. Almeida, M. M. dos Santos, N. G. M. Lima, T. A. Cidral, M. C. N. Melo, and K. C. Lima, "Prevalence and factors associated with wound colonization by Staphylococcus spp. and Staphylococcus aureus in hospitalized patients in inland northeastern Brazil: a cross-sectional study," BMC infectious diseases, vol. 14, no. 1, pp. 1-8, 2014. DOI: 10.1186/1471-2334-14-328.

[21] W. Norbury, D. N. Herndon, J. Tanksley, M. G. Jeschke, C. C. Finnerty, and S. S. C. o. t. S. I. Society, "Infection in burns," Surgical infections, vol. 17, no. 2, pp. 250-255, 2016. doi: 10.1089/sur.2013.134.

[22] L. Thorlacius-Ussing, H. Sandholdt, A. R. Larsen, A. Petersen, and T. Benfield, "Age-dependent increase in the incidence of Staphylococcus aureus bacteremia, Denmark, 2008–2015," Emerging Infectious Diseases, vol. 25, no. 5, p. 875, 2019. doi: 10.3201/eid2505.181733.

[23] O. E. Akanbi, H. A. Njom, J. Fri, A. C. Otigbu, and A. M. Clarke, "Antimicrobial susceptibility of Staphylococcus aureus isolated from recreational waters and beach sand in Eastern Cape Province of South Africa," International journal of environmental research and public health, vol. 14, no. 9, p. 1001, 2017. DOI: 10.3390/ijerph14091001.

[24] C. A. Bastidas, I. Villacrés-Granda, D. Navarrete, M. Monsalve, M. Coral-Almeida, and S. G. Cifuentes, "Antibiotic susceptibility profile and prevalence of mec A and lukS-PV/lukF-PV genes in Staphylococcus aureus isolated from nasal and pharyngeal sources of medical students in Ecuador," Infection and Drug Resistance, pp. 2553-2560, 2019. doi: 10.2147/IDR.S219358.

[25] J. Fri, H. A. Njom, C. N. Ateba, and R. N. Ndip, "Antibiotic resistance and virulence gene characteristics of methicillin-resistant Staphylococcus aureus (MRSA) isolated from healthy Edible Marine Fish," International journal of microbiology, vol. 2020, 2020. DOI: 10.1155/2020/9803903.

[26] S. S. Khoramrooz et al., "Detection of tetracycline resistance genes, aminoglycoside modifying enzymes, and coagulase gene typing of clinical isolates of Staphylococcus aureus in the Southwest of Iran," Iranian Journal of Basic medical sciences, vol. 20, no. 8, p. 912, 2017. DOI: 10.22038/IJBMS.2017.9114.

[27] S. S. Mahmmod and W. G. AlHadban, "Assessing the prevalence and antibiotic susceptibility patterns of S. aureus bacteria isolated from Iraqi women with vaginosis," Iraqi Journal of Science, vol. 63, no. 10, pp. 4234-4240, 2022. DOI: https://doi.org/10.24996/ijs.2022.63.10.10.

[28] S. M. Hantoosh, "Nasal Carriage of Vancomycin-and Methicillin-Resistant Staphylococcus aureus among Intermediate Students of Urban and Rural Schools of Muthanna Province in Iraq," Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512), vol. 31, no. 1, pp. 102-108, 2022. DOI https://doi.org/10.31351/vol31iss1pp102-108.

[29] R. Timsina, U. Shrestha, A. Singh, and B. Timalsina, "Inducible clindamycin resistance and erm genes in Staphylococcus aureus in school children in Kathmandu, Nepal," Future Science OA, vol. 7, no. 1, p. FSO361, 2020. DOI: 10.2144/from-2020-0092.

[30] S. H. Aubaid, E. S. Falih, and S. Khalid Ibrahim, "Biofilm Formation of Staphylococcus Aureus in Multiple Sclerosis Patients and its Essential Role in the Pathogenicity of the Disease," Journal of Techniques, vol. 4, no. 3, pp. 14-18, 2022. DOI: https://doi.org/10.51173/jt.v4i3.511.