RESEARCH ARTICLE

Phenotypic Detection of Macrolide-Lincosamide-Streptogramin Resistance among *Staphylococcus aureus* and *Staphylococcus epidermidis* in Baghdad, Iraq

Mustafa M. Al-Qaisi, Tuqa S. Al-Salmani*

College of Health and Medical Technology, Middle Technical University, Baghdad, Iraq

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ABSTRACT

Introduction: Staphylococci emerged as the most frequent nosocomial and community-acquired pathogens. Macrolide-lincosamide-streptogramin (MLS) antibiotics resistance was increasing among *Staphylococcus aureus* and *Staphylococcus epidermidis* (*S. epidermidis*) isolates.

Objective: In the present study, the aim was to detect the phenotypic resistance pattern of MLS (constitutive and inducible) among *S. aureus* and *S. epidermidis* isolated from Iraqi patients.

Methods: A total of 120 staphylococcal isolates (60 *S. aureus* and 60 *S. epidermidis*) were isolated from urine, wound swab, blood, and sputum specimens, then specified by the VITEK 2 system. Whole isolates were investigated by the disk-diffusion method against many antibiotics, then they were checked for the MLS phenotype by the D-zone test.

Results: Out of 60 *S. aureus* isolates and 60 *S. epidermidis*, the isolation rates from wound, urine, blood, and sputum were 66.6 and 50%, 16.7 and 26.7%, 11.7 and 18.3%, and 5% for each species, respectively. The higher frequency rates of resistance were showed against erythromycin, clindamycin, and streptomycin, for both *S. aureus* with 83.3, 53.3, and 83.3%, respectively, and *S. epidermidis* with 73.3, 45, and 76.7%, respectively. Constitutive MLS resistance phenotype (MLS_c) was shown in 32 isolates (53.3%) of *S. aureus* and inducible MLS resistance phenotype (MLS_i) was noted in 16 isolates (26.7%).

Conclusion: The current study concluded that the D-zone test must be applied within the routine work of the antimicrobial susceptibility test for staphylococcal isolates, to exclude the false results of staphylococcal isolates sensitivity against clindamycin.

Keywords: Constitutive MLS resistance, Inducible MLS resistance, *Staphylococcus aureus*, *Staphylococcus epidermidis*.

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INTRODUCTION

Staphylococci are considered threatening bacteria for all mankind by leading to the community- and hospital-acquired infections.[1,2] *S. aureus* gives rise to several infections for mankind (soft-tissue skin infections, pneumonia, septicemia, endocarditis, and toxic shock syndrome).[3] Besides, *S. epidermidis* also emerged as a dangerous bacteria in vascular catheters, bloodstream, and prosthetic devices infections.[4] The increased spreading of antimicrobial resistance among *S. aureus* [mostly, methicillin-resistant *S. aureus* (MRSA)] and most *S. epidermidis* strains leading to medication failure of infections.[5,6]

The MLS antibiotics act as an inhibitor for protein synthesis by binding to the 23S rRNA ribosomal subunit of bacteria.[7] They are used for treating all gram-positive infections, especially staphylococcal infections.[8] These antibiotics are considered one of the few alternative options for treating MRSA infections.[9] The excessive and improper utilization of MLS antibiotics leads to the increasing development of MLS antibiotic resistance among *S. aureus* and *S. epidermidis* strains.[10,11]

The resistant mechanisms of MLS antibiotics divided into three main routs, including: i) drug inactivation that encoded by *lun* gene, ii) activation of efflux pump by *msr* gene, and iii) alteration of the binding site by methylation of 23S rRNA, which encoded by *erm* genes (*ermA*, *ermB*, *ermC*, and *ermF*).[12-14] The MLS phenotypic resistance includes two: MLS_c or MLS_i.[15] The MLS_c resistance means resistance to erythromycin

*Author for Correspondence: tuqa.sami86@gmail.com

and clindamycin, while the MLS_i resistance is the real resistance to erythromycin and clindamycin, but clindamycin appears susceptible by antimicrobial susceptibility tests.[16] Identification of MLS_i resistance cannot be done by routine antibiotic susceptibility tests, and requires a special test called the D-test method.[17]

Clindamycin is an alternative treatment for penicillin-allergic patients.[18] In patients with MLS_i resistance, treating with clindamycin leads to the development of MLS_c resistance pattern among causative bacteria, then leads to treatment failure.[15] Thus, it is very important to confirm the MLS resistance pattern of causative strains to choose the right treatment.[19] The present study aims to determine the phenotypic resistance pattern of MLS antibiotics as constitutive and inducible among *S. aureus* and *S. epidermidis* isolated from Baghdad in Iraq.

MATERIALS AND METHODS

Samples Collection

One hundred-twenty staphylococci isolates (60 *S. aureus* and 60 *S. epidermidis)* were isolated from different clinical specimens (urine, wound swab, blood, and sputum) collected from patients admitted and visited the Ibn-Albaladi Hospital and Child Welfare Teaching Hospital during the period (November 2018 to May 2019).

Bacterial Identification

The bacterial strains were characterized to species level by different standard microbiological and biochemical tests,[20] and automated by the VITEK-2 system.

Antibiotic Susceptibility Test

All *S. aureus* and *S. epidermidis* were investigated by disk-diffusion method (Kirby-Bauer method) against the following antibiotics: erythromycin (15 µg), clindamycin (2 µg), streptomycin (5 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), tetracycline (30 µg), gentamicin (10 µg), amikacin (30 µg), chloramphenicol (30 µg), nitrofurantoin (300 µg), and vancomycin (30 µg). The results were dependent on the Clinical and Laboratory Standards Institute (CLSI), 2014.[21]

D-zone Test

D-test was utilized to determine the phenotype of MLS resistance as constitutive or inducible for staphylococcal isolates. In inducible MLS resistance, staphylococcal isolates are resistant to erythromycin and susceptible to clindamycin, and lead to the formation of an inhibition zone as D-shape around the clindamycin disk and flattening towards erythromycin. In constitutive MLS resistance, the isolates resistant to both erythromycin and clindamycin, and the inhibition zone appeared spherical.[22,23]

D-test was applied by spreading bacterial suspension (equivalent to 0.5 McFarland's standard) on Mueller-Hinton media, then clindamycin disk (2 µg) was placed at a distance of 15 mm from erythromycin (15 µg), then incubated at 35ºC overnight, as recommended by CLSI, 2012.[24] The results of D-test were divided into four phenotypes, as follows[10,25]:

- *MLS_i-inducible:* The isolates showed resistance to erythromycin, while the clindamycin disk appeared as a D-shape zone around it.
- *MLS_c-constitutive:* The isolates were resistant to erythromycin and clindamycin.
- *MS-moderate sensitive:* The isolates appeared resistant to erythromycin and susceptible to clindamycin without a D-shaped zone.
- *S-sensitive:* The isolates were sensitive to both erythromycin and clindamycin.

RESULTS

Bacterial Isolation

A total of 120 staphylococcal isolates (60 *S. aureus* and 60 *S. epidermidis*) were isolated from different specimens. Table 1 showed the frequency of staphylococcal isolates among clinical specimens. The highest isolation rate of *S. aureus* was isolated from an infected wound with 66.6% (40 isolates). *S. aureus* isolates also isolated from urine and blood with isolation rates 16.7% (10 isolates) and 11.7% (7 isolates), respectively. From sputum, *S. aureus* recorded lower isolation rate with 5% (only 3 isolates).

On the contrary, *S. epidermidis* also showed that the higher isolation rate was from wound samples with 50% (30 isolates), while the lower isolation rate was from sputum samples with 5% (3 isolates). The isolation from urine and blood samples were 26.7 and 18.3%, respectively, as shown in Table 1.

Antimicrobial Susceptibility Patterns

In *S. aureus* isolates, the higher percentage of resistance was showed against erythromycin and streptomycin with 83.3% (50 *S. aureus* isolates), and *S. epidermidis* was 73.3% (44 isolates) and 76.7% (46 isolates), respectively. Fluoroquinolones (ciprofloxacin and norfloxacin) came in the second step with a percentage of resistance 68.3% (41 *S. aureus* isolates) and 60% (36 *S. epidermidis* isolates), as shown in Table 2. The resistance rate of

Table 1: Distribution of staphylococcal isolates according to clinical specimens

Specimens	*No. of isolates (n = 120)*	*S. aureus (n = 60)*		*S. epidermidis (n = 60)*	
		No.	*%*	*No.*	*%*
Urine	26	10	16.7	16	26.7
Wound	70	40	66.6	30	50
Blood	18	7	11.7	11	18.3
Sputum	6	3	5	3	5

Table 2: Antimicrobial susceptibility patterns among *S. aureus* and *S. epidermidis* isolates

	S. aureus (n = 60)		*S. epidermidis (n = 60)*	
Antibiotic	*No.*	*%*	*No.*	*%*
Erythromycin	50	83.3	44	73.3
Clindamycin	32	53.3	27	45
Streptomycin	50	83.3	46	76.7
Cefoxitin	32	53.3	28	46.7
Ciprofloxacin	41	68.3	36	60
Norfloxacin	41	68.3	36	60
Tetracycline	27	45	30	50
Gentamicin	30	50	30	50
Amikacin	24	40	28	46.7
Chloramphenicol	26	43.3	22	36.7
Nitrofurontine	11	18.3	9	15
Vancomycin	19	31.7	7	11.7

Table 3: Distribution of MLS resistance phenotypes among *S. aureus* and *S. epidermidis* isolates

	MLS phenotypes			
Bacterial isolates	*[a]MLS_c No. (%)*	*[b]MLS_i No. (%)*	*[c]MS No. (%)*	*[d]S No. (%)*
S. aureus (n = 60)	32 (53.3%)	16 (26.7%)	2 (3.3%)	10 (16.7%)
S. epidermidis (n = 60)	27 (45%)	14 (23.3%)	3 (5%)	16 (26.7%)

[a]MLS_c = Constitutive MLS resistance phenotype; [b]MLS_i = Inducible MLS resistance phenotype; [c]MS = Moderate sensitive phenotype (erythromycin resistance and clindamycin sensitive); [d]S = Sensitive phenotype (both erythromycin and clindamycin sensitive)

clindamycin was 53% (32 of *S. aureus*) and 45% (27 of *S. epidermidis*). Among 32 isolates of *S. aureus*, the isolation rate of MRSA was 53% depending on the cefoxitin resistance, while in *S. epidermidis*, the isolation rate of cefoxitin resistance was 46.7% (28 isolates). Additionally, vancomycin-resistant *S. aureus* (VRSA) showed in 19 isolates (31.7%) of *S. aureus* and in 7 isolates (11.7%) of *S. epidermidis*. The resistance pattern to tetracycline, gentamicin, amikacin, and chloramphenicol showed a resistance rate of 45, 50, 40, and 43.3%, respectively, for *S. aureus* isolates, and 50, 50, 46.7, and 36.7%, respectively, for *S. epidermidis*. Nitrofurontine recorded low resistance levels in both *S. aureus* and *S. epidermidis* isolates with 18.3 and 15%, respectively.

MLS Phenotypes

In the current study, the results in Table 3 show the distribution of MLS phenotypes among *S. aureus* and *S. epidermidis*. These results show that out of 60 *S. aureus* isolates, 32 isolates (53.3%) showed MLS_c resistance phenotype. MLS_i resistance phenotype was seen in 16 isolates (26.7%). Two isolates (3.3%) showed moderate resistance phenotype (MS), which were resistant to erythromycin and sensitive to clindamycin. Ten of *S. aureus* isolates showed a sensitivity phenotype.

The MLS resistance phenotypes were determined for 60 *S. epidermidis* isolates. As shown in Table 3, 27 (45%) of isolates showed MLS_c resistance phenotype and 14 (23.3%) of isolates showed MLS_i resistance phenotype. MS resistance phenotype was shown in just 3 isolates, while the S resistance phenotype was shown in 16 isolates.

Antimicrobial Resistance Pattern among MLS_c and MLS_i Resistance Phenotypes

The outcomes in Table 4 show the antimicrobial resistance profile, according to MLS_c and MLS_i resistance phenotypes of both *S. aureus* and *S. epidermidis*. In *S. aureus*, 25 (78.1%) out of 32 MLS_c resistant isolates were MRSA, and in 4 (25%) out of 16 MLS_i resistant isolates. 16 isolates (50%) of MLS_c resistant isolates and 3 (18.3%) of MLS_i resistant isolates were VRSA.

Among MLS_c resistant *S. aureus* isolates, the higher resistance rates were recorded in 20 isolates (62.5%) against several antibiotics, including ciprofloxacin, norfloxacin, gentamycin, and amikacin, and also the high resistance rates were recorded in 17 isolates (53.1%) and 15 isolates (46.9%) against chloramphenicol and tetracycline, respectively. A low resistance rate was shown against nitrofurontine 21.9 (7 MLS_c resistant *S. aureus* isolates).

Among MLS_i resistant *S. aureus* isolates, the highest resistance rate was shown in 14 isolates (87.5%) against fluoroquinolones (ciprofloxacin and norfloxacin). On the contrary, the resistance rates were shown low against amikacin 25%, chloramphenicol 25%, and nitrofurontine 6.3%. Tetracycline and gentamicin resistance rates were 43.8 and 37.5%, respectively, among MLS_i resistant *S. aureus* isolates, as mentioned in Table 4.

In *S. epidermidis*, cefoxitin resistance was shown in 18 (66.7%) out of 27 MLS_c resistant isolates and in 7 (50%) out of 14 MLS_i resistant isolates, while vancomycin resistance was shown in 5 (18.5%) of MLS_c resistant isolates, and in just 1 (7.1%) of MLS_i resistant isolates. In MLS_c

Table 4: Antimicrobial resistance profile among MLS_c and MLS_i resistance phenotypes of *S. aureus* and *S. epidermidis* isolates

	S. aureus		*S. epidermidis*	
Antibiotic	[a]*MLS_c (n = 32)* *No. (%)*	[b]*MLS_i (n = 16)* *No. (%)*	*MLS_c (n = 27)* *No. (%)*	*MLS_i (n = 14)* *No. (%)*
Cefoxitin	25 (78.1)	4 (25)	18 (66.7)	7 (50)
Ciprofloxacin	20 (62.5)	14 (87.5)	20 (74.1)	9 (64.3)
Norfloxacin	20 (62.5)	14 (87.5)	20 (74.1)	9 (64.3)
Tetracycline	15 (46.9)	7 (43.8)	18 (66.7)	7 (50)
Gentamicin	20 (62.5)	6 (37.5)	20 (74.1)	8 (57.1)
Amikacin	20 (62.5)	4 (25)	20 (74.1)	8 (57.1)
Chloramphenicol	17 (53.1)	4 (25)	12 (44.4)	7 (50)
Nitrofurontine	7 (21.9)	1 (6.3)	6 (22.2)	2 (14.3)
Vancomycin	16 (50)	3 (18.8)	5 (18.5)	1 (7.1)

[a]MLS_c = Constitutive MLS resistance phenotype; [b]MLS_i = Inducible MLS resistance phenotype

resistant *S. epidermidis* isolates, the higher resistance rates were shown in 20 isolates (74.1%) against ciprofloxacin, norfloxacin, gentamycin, and amikacin. The high resistance rates were shown in 18 isolates (66.7%) and 12 isolates (44.4%) against tetracycline and chloramphenicol, respectively. Nitrofurontine resistance was low and it was shown in 6 (22.2%) of MLS_c resistant *S. epidermidis* isolates, as shown in Table 4.

In MLS_i resistant *S. epidermidis* isolates, the high resistance rates were shown in 64.3% (9 isolates) against ciprofloxacin and norfloxacin, 57.1% (8 isolates) against gentamicin and amikacin, and 50% (7 isolates) against tetracycline and chloramphenicol. On the contrary, the lower resistance rate was shown against nitrofurontine with 14.3% (2 of MLS_i resistant *S. epidermidis* isolates).

DISCUSSION

Clindamycin is an important antibiotic used to treat several serious infections caused by *Staphylococcus* species, particularly MRSA isolates. The misuse or excessive use of this agent leads to development of resistance, eventually therapy failure. Previous studies reported this misuse, where clindamycin was utilized in treating infections caused by MLS_i resistance staphylococcal isolates.[26,27] This happens due to the misdiagnosis of the clindamycin susceptibility test. Although the D-zone test is an important, very easy, and low-cost test, it is not used in our laboratories during routine work. This may lead to the development of clindamycin resistance or MLS_c resistance phenotype among causative agents.

In our study, the highest isolation rate of *S. aureus* from samples was isolated from an infected wound with 66.6%. This outcome was higher than that reported by another local study carried by Mahdi *et al.*, 2020 that reported an isolation rate of 41% of *S. aureus* isolates.[28] Besides, this result was also higher than that in other studies, although these studies also recorded the highest isolation rate of *S. aureus* from the wound.[29,30] The lowest isolation rate of *S. aureus* were collected from sputum sample with 5%. This data was identical to that reported by Pournajaf *et al.*,[31] Khodabandeh *et al.*,[29] and Mohammadia *et al.*[30] Additionally, the isolation rate of *S. epidermidis* was highest collected from wound 50%, while, from blood 18.3%, these results were in contrast with those reported by another study that showed isolation rate 13.3% from the wound and 36.6% from the blood.[32]

The results of the antimicrobial susceptibility test by routine disk diffusion method showed that the highest antimicrobial resistance rate was against erythromycin 83.3% of *S. aureus* and clindamycin 53.3% of isolates, these results are close to those reported by previous studies.[29,33] In *S. epidermidis*, the resistance rates for erythromycin and clindamycin were 73.3 and 45%, respectively. These outcomes were lower than those reported by another study.[32] Thirty-two *S. aureus* isolates (53%) were MRSA; this result is lower than that reported in another local study, carried in Al-Sulaimania city that showed 68% of *S. aureus* isolates were MRSA isolates.[34] While, the results were higher than those reported by another regional study carried in Iran by Nikbakht *et al.*, who showed the isolation rate of MRSA approximately 39%,[35] while in *S. epidermidis*, the percentage of cefoxitin resistance was 46.7%. This result is lower than that reported in another study that showed a high rate of MRSA 93%.[34]

Additionally, VRSA showed in 19 isolates (31.7%) of *S. aureus*[33] and among *S. epidermidis* isolates, 11.7% of isolates showed vancomycin resistance. This result is close from that reported by another local study that recorded a vancomycin resistance rate 10%,[32] but this result is far from this obtained by another local study that reported a resistance rate 40%.[36] In general, the results mentioned in Table 3 obtained by utilizing the D-zone test, because this test gives true results of inducible clindamycin resistance. The results showed that the most frequent resistance phenotype among staphylococcal isolates (*S. aureus* and *S. epidermidis*) was MLS_c with 53.3 and 45%, respectively, and the second most frequent resistance phenotype was MLS_i (26.7 and 23.3%, respectively). MS phenotype showed in just 3.3% of *S. aureus* and 5% of *S. epidermidis* isolates, as shown in Table 3. These data differed from that reported by another study that showed MLS_i phenotype was the most common among staphylococcal

isolates, MS phenotype came in the second step and MLS_c phenotype was shown in only 8.9% of staphylococcal isolates.[37]

In *S. aureus*, the frequency of MLS_c and MLS_i resistance phenotypes was 53.3 and 26.7%, respectively. These results were near to the study performed by Khodabandeh *et al*.,[29] while they were far and disagree with other studies carried by Ghanbari *et al*.[38] and Moosavian *et al*.[39] Among *S. epidermidis*, 45% of isolates showed MLS_c resistance phenotype and 23.3% of isolates showed MLS_i resistance phenotype. MS resistance phenotype was shown in just 5% of isolates. These results disagree with another study conducted that the frequency of MLS_c, MLS_i, and MS resistance phenotypes were in order 16.7, 83.3, and 0%, respectively.[13] Out of 32 MLS_c and 16 MLS_i resistant *S. aureus* isolates, 25 (78.1%) and 4 (25%) were MRSA, while 60 (50%) of MLS_c resistant isolates, and 3 (18.3%) of MLS_i resistant isolates were VRSA (Table 4). In MLS_c and MLS_i resistant *S. epidermidis* isolates, the isolation rate of MRSA isolates were 66.7 and 50%, respectively, and VRSA isolates were 18.5 and 7.1%, respectively. The frequency rate of MRSA among MLS_i resistant *S. aureus* and *S. epidermidis* were high. These results were considered not perfect because all these isolates appeared to have a clindamycin sensitivity pattern by routine disk diffusion method, but it has really appeared resistant on D-test. Most of these isolates were treated with clindamycin. Eventually, this will lead to an increase in the MLS_c resistance rate in the future. In this study, these results may explain why the higher MLS_c resistance rate has appeared among *S. aureus* and *S. epidermidis* isolates. From these results, D-test should be applied as a routine work in laboratories to determine if the staphylococcal isolates are really sensitive for clindamycin or not.[39]

CONCLUSION

In this study, the rate of MLS_c resistance phenotype was highest among *S. aureus* and *S. epidermidis* isolates, and the rate of MLS_i resistance phenotype was also high among both species. On the contrary, the rate of MRSA among MLS_i resistant for both species was high and clindamycin commonly used in treating MRSA infections. This clarifies the highest rate of MLS_c resistance phenotype among isolates that result from misdiagnosis of the clindamycin sensitivity profile. This study concluded that the D-zone test should be applied within the routine work of the antimicrobial susceptibility test for staphylococcal isolates in microbiology laboratories to exclude the false results of staphylococcal isolates sensitivity against clindamycin.

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