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Study the Expression of *msrA,msrB* and *linA/linA'* genes in Presence of Some Antibiotics in Methicillin Resistance *Staphylococcus aureus*

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Abstract

Eight isolates of methicillin resistance *Staphylococcus aureus*(MRSA) (SA40,SA32,SA30,SA13,SA10,SA36,SA3 and SA7) with different resistance phenotypes to macrolides , lincosamides and streptogramins Were used to detect theexpression of *msrA, msrB,* and *linA/linA'*genesby using real time polymerase chain reaction before and after treatment with antibiotics (erythromycin , clarithromycin , clindamycin and lincomycin) calibrated with triosphosphateisomerase.There highst expression of these genes was after 18 hours. It was an induction in the expression of *msrA* gene in isolates (SA40,SA32,SA30 and SA13) in presence of erythromycin,however,the isolates showed reduction in expression level of this gene .

Expression of *msrB* gene had been estimated along with the using of clarithromycin and there was induction in *msrB* expression in isolates (SA40,SA30,SA32,SA13,SA36,SA10and SA7) while the expression level was reduced in isolate (SA3). Isolates (SA30 and SA13) showed induction of *linA/linA'* expression with the using of clindamycin and lincomycin respectively.

Keywords: MRSA,MLS resistance phenotypes,*msrA,msrB* and*linA/linA'*

دراسة التحري عن التعبير الجيني لجيناتmsrA و msrB و linA/linA' بوجود بعض المضادات في المكورات العنقودية المقاومة للمثيسيلين

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الخلاصة

استخدمت ثمان عزلات من بكتريا المكورات العنقودية المقاومة للمثيسيلين SA40 ,SA32,SA30,SA13,SA10,SA36,SA3و SA7وبمختلف الانماط المظهرية المقاومة لمضادات الماكروليدات واللنكوسأميد والستربتوغرامين للتحري عن التعبير الجيني لجيناتmsrA و msrB و linA/linA' بوساطة تفاعل البلمرة الكمي اللحظي قبل وبعد المعاملة مع المضادات (الاريثرومايسين, كلاريثرومايسين, كليندامايسين و اللنكومايسين) وبالموازنة مع جين ترايوز فوسفات ايزوميراز.اعلى تعبير جيني كان بعد 18 ساعة . كان هناك تحفيز بالتعبير الجيني لجين *msrA*للعزلات(SA40,SA32,SA30 و SA13) بوجود مضاد الاريثرومايسين والعزلات الباقية اظهرت تثبيط في مستوى التعبير الجيني لهذا الجين . تم حساب التعبير الجيني لجين *msrB*بأستخدام مضاد الكلاريثرومايسين , كان هناك تحفيز بالتعبير الجيني في العزلات(SA40,SA30,SA32,SA13,SA36, SA10 و SA7) بينما ثبط التعبير الجيني للعزلةSA3 .

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اظهرت العزلتين (SA30 و SA13) تحفير في تعبير الجين *linA/linA'* بأستخدام مضادي كليندامايسين و اللنكومايسين تباعاً

Introduction

Staphylococcus aureus is both commensal organism and also an important opportunistic human pathogen, causing a variety of community and hospital-associated infections, such as bacteremia,sepsis,endocarditis, pneumonia, osteomyelitis, arthritis and skin diseases [1] .Antimicrobial resistance is one of the most serious health threats ,infections from resistantbacteria are now very common and some pathogens have even become resistant tomultiple types or classes of antibiotics, *S. aureus* become a major public health concern as a result of the steadily increasing incidence of antimicrobial resistance particularly methicillin resistant*S. aureus* (MRSA) [2].MRSA is a serious problem in the treatment and control as as aresult of multidrug resistantandability to cause wide variety of human diseases [3-4]. MRSA have resistance to many commonly used groups of antibiotics like beta lactams, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, and tetracycline[5].The macrolide lincosamide and streptogramin (MLS) family of antibiotics were first introduced in 1952act as an alternative therapeutic agent especially with penicillin allergic patients ,MLS group of antibiotics have the same target which is the bacterial 50S ribosomalsubunit, thereby effectively inhibiting proteinsynthesis [6] .However, resistance to the these antibioticsemerges shortly afterwards in *S. aureus*as resistance genes are already present.Theusing of these antibiotics cause a selectiveefflux pump of the antibiotics out of the bacterial cell before reaching the ribosome [7]. Efflux pump is a primary defensemechanism and it is quite common in some geographical areas encodes by *msrA* and *msrB*genesin*S.aureus* [8]. *msrA*responsible for resistance to MLS antibiotics[9].Genes encoding efflux pumpsfound to be part of the normal genetic makeup of *S. aureus* and other human pathogens, resistance to a number of antimicrobials occure when these genes are overexpressed [10].Lincosamides (clindamycin and lincomycin) are useful drugs for treatment of infections caused by MRSA, but change in clindamycin sensitivity pattern due to various mechanisms leading to therapeutic failure [11]. Resistance to clindamycin either constitutive caused by *linA/linA'* through enzymatic inactivation mechanism or induced by other mechanisms like efflux pump and target modification [12]. Resistance of staphylococci tostreptogramins antibiotics encodes by*vga*and causing active efflux of streptogramin in staphylococci [13].

MLS Resistance phenotypes are :

-M Phenotype : Staphylococcal isolates exhibiting resistance to erythromycin which they are sensitive to clindamycin and giving circular zone of inhibition around clindamycin [14]. Figure-1(a).

-Inducible MLS (iMLS) Phenotype : Staphylococcal isolates showresistance to erythromycin and clindamycin giving D shape zone of inhibition around clindamycin with flattening towards erythromycin[15]. Figure-1(b).

-Constitutive MLS(cMLS) Phenotype : resistant to macrolide, lincosamide and streptograminantibiotics, this phenotype detects for those staphylococcal isolates that are showing resistance to both erythromycin and clindamycin [16]. Figure-1(c).

- SAB : resistance to streptogramins A and B antibiotics (Leclercq and [17].

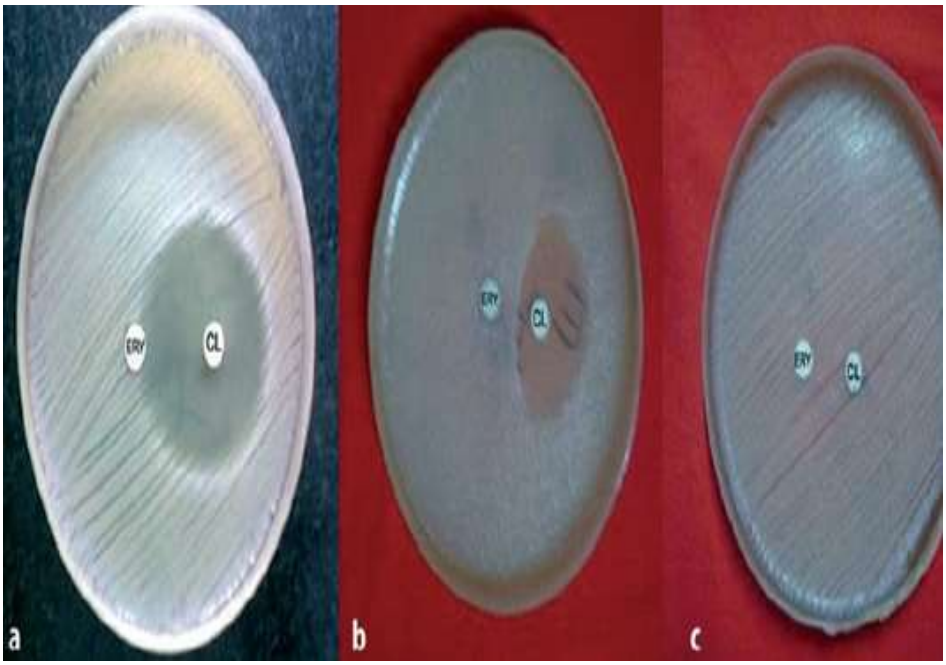

Figure 1-MLS resistance phenotypes (a): M Phenotype ,(b): Inducible MLS (iMLS) Phenotype ,(c) Constitutive MLS (cMLS) Phenotype , ERY:Erythromycin , CL: Clindamycin [18-19].

Isolation and Identification of staphylococci

Isolation of staphylococci from different clinical specimens by specific way depending on routine laboratory techniques. Isolates identified by biochemical test and confirmed by Vitek2 compact systesm , MRSA isolate determined with disc diffusion test by using the antibiotics oxacillin and cefoxitin .

MLS resistance phenotypes

MLS resistance phenotypes determined by using clindamycin and erythromycin antibiotic discs with disc diffusion test ,these tests involve the placement of an erythromycin disc in close proximity (10-15) mm to a disc containing clindamycin or lincomycin.

RNA Extraction from *S.aureus* isolates by using Wizard®SV total RNA isolation system

1-Isolates (SA40,SA32,SA30,SA13,SA10,SA36,SA3 and SA7) were cultured on mannitol salt agar .After incubation for 24 hrs. at 37^{o}C, the bacterial isolates were inoculated in tubes contained 5 ml of sterile Brain-Heart infusion broth with antibiotics at concentrationsbelow MIC values(Clindamycin,Lincomycin, Erythromycin and Clarithromycin) and incubated for 18 hrs. at 37 ^{o}C. For every isolate there were two tubes for the tested gene and house keeping gene.

2-Calibrators prepared for both of tested genes and house keeping gene byinoculatedbacterial isolates in tubes contained 5 ml of sterile Brain-Heart infusion broth with out antibiotics then incubated for 18 hrs. at 37 ^{o}C.

3-From bacterial growth , 1 ml was transferred to a 1.5 ml microcentrifuge tubes and centrifuge at 14000 g for 2 min.to pellet the cells, the supernatant was removed.

4-The pelleted cells were resuspendedin 100µl of TE buffer and 20 µl of lysozyme, then mix in vortex.

5-The microcentrifuge tubes were put in water bath for 30 min. at 37 ºC.

6-RNA lysis buffer (RLA) 74 µl and RNA dilution buffer (RAD) 350 µl were added to the tubes , waited for 5 min. and centrifuged gently at 12000g for 1 min.

7-Absolute ethanol 200 µl were added to each tube .

8-RNA solutions were removed from microcentrifuge tubes to filter tubes , then centrifuge at 14000 g for 2 min .

9-RNA wash solution 600 µl was added .

10-DNase solution50 µl was added , placed in room temperature for 15 min.

11-DNase stop solution 200µl was added and centrifuge at 14000 g for 1 min.

12-RNA wash solution 600 µl was then added and centrifuged at 14000 g for 1 min.

13-RNA wash solution 250 µl was added after that and centrifuged at 14000g for 2 min.

14-Filter tubes were removed from their tubes to microcentrifuge tubes then 100 µl of sterilenuclease free water was added and waited for 5 min.andthen centrifuged at 14000 g for 1 min.
15-RNA solution was dissolved in water and get down in microcentrifuge tubes.16-The RNA was stored in -20 ^{o}C.

Quantitative reverse transcription-PCR (1-Step qRT-PCR)

Thehousekeeping gene triosephosphateisomerase (*tpi*)was selected for this study. This primer with other primers under this study were provided in a lyophilized form (Promega) and dissolved insterilenuclease free waterto give a final concentration of 100pmol⁄µl and stored in deep freezer until used in qPCRamplification,Table-1.

Table 1-The primerand their sequence used in qPCR amplification

Target gene	Primer name	Primer sequences5-3	PCR frag-ment size(bp)	References
msrA	MsrA F	**GGCACAATAA GAGTGTTTAA AGG**	**940**	[20]
msrA	MsrAR	**AAGTTATATC ATGAATAGAT TGTCCTGTT**		
msrB	MsrB F	**TATGATATCC ATAATAATTA TCCAATC**	**595**	[20]
msrB	MsrB R	**AAGTTATATC ATGAATAGAT TGTCCTGTT**		
linA/linA'	linA/linA′ F	**GGTGGCTGGG GGGTAGATGT ATTAACTGG**	**323**	[21]
linA/linA'	linA/linA′ R	**GCTTCTTTTG AAATACATGG TATTTTTCGA TC**		
tpi	tpiF	GGTCATTCTGAACGTCGTGA	-	[22]
tpi	tpiR	TGATAAACGATACGTCCTGCAC	-	

The extracted RNA, primers and qPCR premix (Go Taq 1-Step qRT-PCR System) were thawed at 4^{o}C. The thowedmixuture were vortexed then centrifuged briefly to bring the contents to the bottom of the tubes. PCR mixture was set up in a total volume of 25µl included 12.5µl of PCR premix, 0.5µl of reverse transcriptase mixture , 2.5µl of each primer, 5 µl of RNA have been used andthe rest volume was completed with sterile nuclease free water.qPCR premixes were prepared for *tpi* primer, *msrA*,*msrB* and *linA/linA'* primers in two replica for each one.qPCR reaction tubes placed in to the thermocyclerqPCR instrument where RNA expression was indicated as shown in Table-2.The expression of the targeted genes was quantified by using the SYBR green reagent in (1-Step qRT-PCR Kit Promega / USA).

PCR was performed in optimized conditions Tables-(3,4,5) and the fluorescence signals were measured over 40 PCR cycles,the cycle number (Ct) at which the signals crossed a threshold set within the logarithmic phase was recorded.

Expression levels were quantified using relative quantitation.The difference in cycle thresholds (ΔCt) and fold changes evaluated between the treated groups and calibrators of each gene [23].These values were normalized to *tpi*expression as showed below :

ΔCt = Ct of tested gene-Ct of house keeping gene

ΔΔCt = ΔCt (sample) – ΔCt (calibrator)

Fold changes = $2^{-\Delta\Delta Ct}$

Sample: tested genes and house keeping gene treated with antibiotics

Calibrator:tested genes and house keeping gene with out antibiotics

Table 2-Mastermix components for 1-Step qRT-PCR

Reagents	Volume (µl)
Nuclease free water	2
Reverse transcriptase mixture	0.5
Forword Primer (10 µM)	2.5
Reverse Primer (10 µM)	2.5
Template Genomic RNA	5
Go Taq 1-Step qRT-PCR	12.5
Total volume	25

Table 3-Reaction conditions for 1-Step qRT-PCR for *msrA*and*msrB*

Stage	Temperature (time)	
Reverse transcriptase	37°C (15min.)	
Hot start	95°C (10min.)	
Denaturation	95°C (30sec)	40 cycles
Annealing	50°C (30sec)	
Extension	72°C (30sec)	
Melting curve	60-95°C	

Table 4-Reaction conditions for 1-Step qRT-PCR for *linA/linA'*gene.

Stage	Temperature (time)	
Reverse transcriptase	37°C (15min.)	
Hot start	95°C (10min.)	
Denaturation	95°C (30sec)	40 cycles
Annealing	57°C (30sec)	
Extension	72°C (30sec)	
Melting curve	60-95°C	

Table 5-Reaction conditions for 1-Step qRT-PCR for *tpi*gene.

Stage	Temperature (time)	
Reverse transcriptase	37°C (15min.)	
Hot start	95°C (10min.)	
Denaturation	95°C (30sec)	40 cycles
Annealing	64°C (30sec)	
Extension	72°C (30sec)	
Melting curve	60-95°C	

Results and Discussion

Eight isolates of *S.aureus* Which were SA40 , SA32 ,SA30 , SA13,SA10 , SA36,SA3 and SA7 isolate from different clinical specimens , all of them were MRSA.

MLS resistance phenotypes:

The results showed that there were four types of MLS phenotypes iMLSB resistance ,cMLS resistance, M phenotype and SABresistance phenotype as showed in Table-6.

Table 6-No. of *S.aureus* isolates and their MLS resistance phenotypes

Isolate	MLS resistance phenotype
SA40 and SA32	iMLSB resistance
SA30 and SA13	cMLS resistance
SA10 and SA36	M
SA3 and SA7	SAB

as the erythromycin diffuses through the agar, resistance to the lincosamide induces , resulting in a flattening or blunting of the lincosamide zone of inhibition adjacent to the erythromycin disk giving a D shape to the zone (D-zone effect) [24-25]. D-zone may be positive results for detection of inducible clindamycin resistance in *S.aureus* showing blunting inhibition zone around clindamycin disc or a negative D-zone showing no blunting inhibition zone around clindamycin disc [26].

RNA from *S.aureus* isolates

RNA was extracted from 8 isolates of *S.aureus* with different MLSresistance phenotypes by using SV total RNA isolation system to study the expression of MLS resistance genes *msrA,msrB* and *linAl/linA'* in the presence of the house keeping gene thetriosephosphateisomerase (*tpi*) which was responsible for glucose metabolismby catalyzed the isomerization of glyceraldehydes 3-phosphate (G3P) and dihydroxy-acetone phosphate (DHAP) in glycolysis and gluconeogenesis[22].Erythromycin, clarithromycin, lincomycin andclindamycin, were used to study the expression of MLS resistance genes which was calculated by fold changes.

Effect of erythromycin on expression of *msrA* gene

In order to determine the effect of erythromycin on the expression of *msrA* gene in *S.aureus* isolates after 18 hrs.of exposure time , qRT-PCR was used to quantify *msrA* mRNAexpression in isolates. The results showed that there was induction in the expression of *msrA*(fold changes were 10.6 ,11.3 ,5.3 ,3.7) for isolates SA40 SA32, SA13 and SA30 which had resistance phenotypes to erythromycin at MIC level 8 µg/ml ,Figures-(2, 3) and Table-7.

There was induction in *msrA* expression in these isolates atconcentrationbelow MIC value (4 µg/ml). *msrA* gene was responsible for the efflux mechanism in staphylococci which was upregulated after exposure to macrolide that led to pump out macrolides and streptogramin B [27].

Reduction in expression of *msrA*was observed in isolates SA10, SA36, SA3 and SA7 atconcentration below MIC value (4 µg/ml)for isolates SA10 and SA36 and (0.1 µg/ml) for isolates SA3 and SA7 which mean the effect of antibiotic in inhibition of mRNA transcription and expression ,it has been shown that various antibiotic classes have effects on gene transcription, upregulation and downregulation of diverse genes such as antibiotics resistance genes and virulence genes with some estimates suggesting that as many as 5% of gene promoters might be affected [28].The *msr*A gene encode a protein (488 amino acid) with 2 ATP-binding domains characteristic of ABC transporters for efflux system appears to be multicomponent in nature, involving *msr*Awhich encode an inducible mechanism of erythromycin resistance and other chromosomal genes to constitute a fully operational efflux pump that has specificity for 14- and 15-membered macrolides and type B streptogramins [20].Erythromycin acts as an inducer of resistance to three different classes of inhibitors of the 50S ribosomal subunit-the macrolides, lincosamides, and streptogramins antibiotics, 50S ribosomal subunits isolated from induced or constitutively resistant cells showed decrease in ability to bind erythromycin and lincomycin [29].Expression of *bpeAB-oprB* induced by its substrate (erythromycin) and upon entry into stationary phase [30]. Other study on expression of *NorA, NorB*and *NorC* efflux pump genes which conferring resistance to quinolones in MRSA showed that the expression occured in early-stationary and stationary-phase after exposure to antibiotic [31].

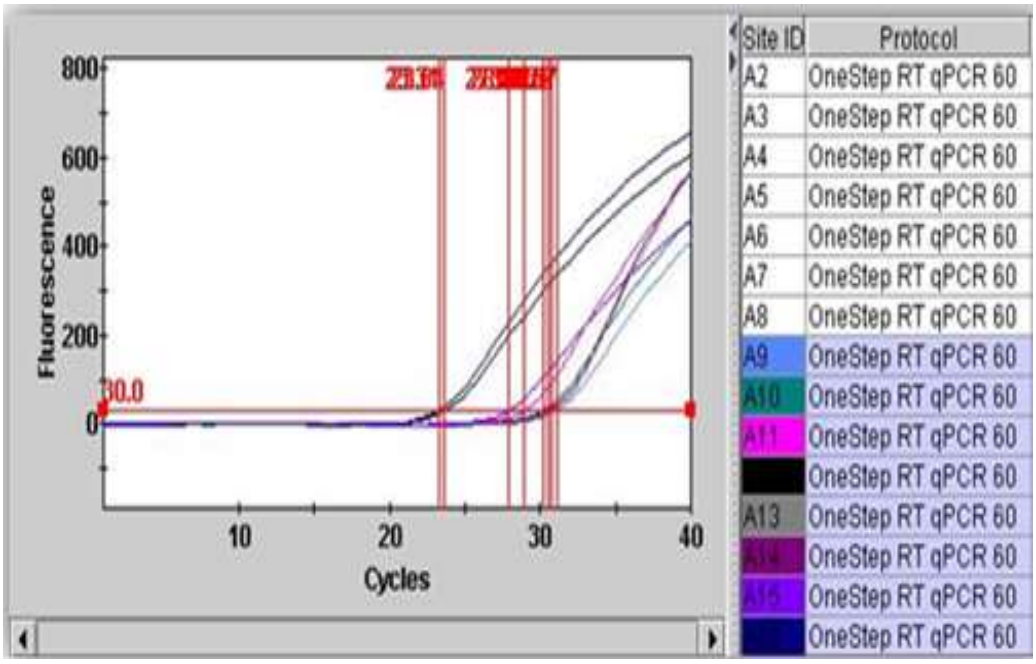

Figure 2-Ct values of *msrA*genein *S.aureus* isolates (SA3, SA13,A10,SA32) (A9:SA3 , A11:SA32, A13:SA10 , A15:SA13): calibrator , (A10:SA3 ,A12:SA32 , A14 :SA10 , A16:SA13): with antibiotic (erythromycin).

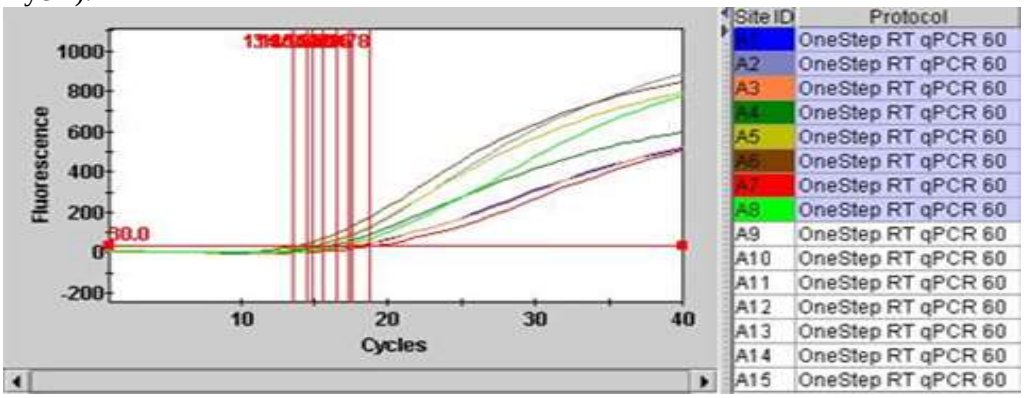

Figure 3-Ct values of *tpi*genein *S.aureus* isolates (SA3, SA13,SA10,SA32), (A1:SA3 , A3:SA32, A5:SA10 , A7:SA13): calibrator , (A2:SA3 ,A4:SA32 , A6 :SA10 , A8:SA13): with antibiotic(erythromycin).

Table 7-Fold changes in expression of *msrA* after treated with erythromycin

	Calibrator			**Erythromycin**				
Sample	**Ct*msrA* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**Ct*msrA* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**ΔΔCt**	**Fold change**
SA40	32.2	17.1	15.1	34.1	22.4	11.7	-3.4	10.6
SA32	28.9	17.4	11.5	23.6	15.6	8	-3.5	11.3137
SA13	27.9	18.8	9.1	23.3	16.6	6.7	-2.4	5.2780
SA30	32.3	17.1	15.2	34.2	20.9	13.3	-1.9	3.7
SA10	30.7	14.5	16.2	30.5	13.6	16.9	0.7	0.6156
SA36	32.3	20.6	11.7	34.3	21	13.3	1.6	0.3
SA3	30	17.6	12.4	30.1	14.9	15.2	2.8	0.1436
SA7	33	18.8	14.2	34.6	17.3	17.3	3.1	0.1

Effect of clarithromycin on expression of *msrB* gene

In order to determine the effect ofclarithromycin on the expression of *msrB*gene in *S.aureus* isolates after 18 hrs.of exposure time , qRT-PCR was performed to quantify *msrB* mRNAexpression in isolates. The results showed that there was induction in the expression of *msrB* in isolates at concentration below MIC value(4µg/ml) the expression levels (fold change) of *msrB* gene in isolates SA40,SA32,SA13,SA30,SA10, SA36 and SA7 which had resistance to clarithromycin were (64, 4.6, 6.5, 1.2 , 3,6 and 27.9) respectively . This mean the role of this antibiotic as inducer of gene expression like erythromycin because these antibiotics in addition to other types of macrolides such as azithromycin are strong inducers in expression of *msrB* gene which caused resistance to these antibioticsby efflux mechanisms which was the most common mechanism of resistance to MLS antibiotics [32].Clarithromycin induce the expression of *msrB* in isolates SA40 which had iMLSresistane phenotype, and highest expression level, this mean that clarithromycin act as inducer for *msrB* gene expression ,resistance mean that the determinant (*msrB*) was active and the expression had been carried out, macrolides resistance genes affected by differents types of antibiotics related to this group like azithromycin, roxithromycin , dirithromycin , isolate SA3 was sensitive to clarithromycin thus there is no expression of *msrB* gene, Figures-(4,5) and Table-8.The highly expression of macrolides resistance genes in 18 hrs. mean that there were a disadvandages with the using of clariyhromycin that was necessary to take multiple daily dosing [33].

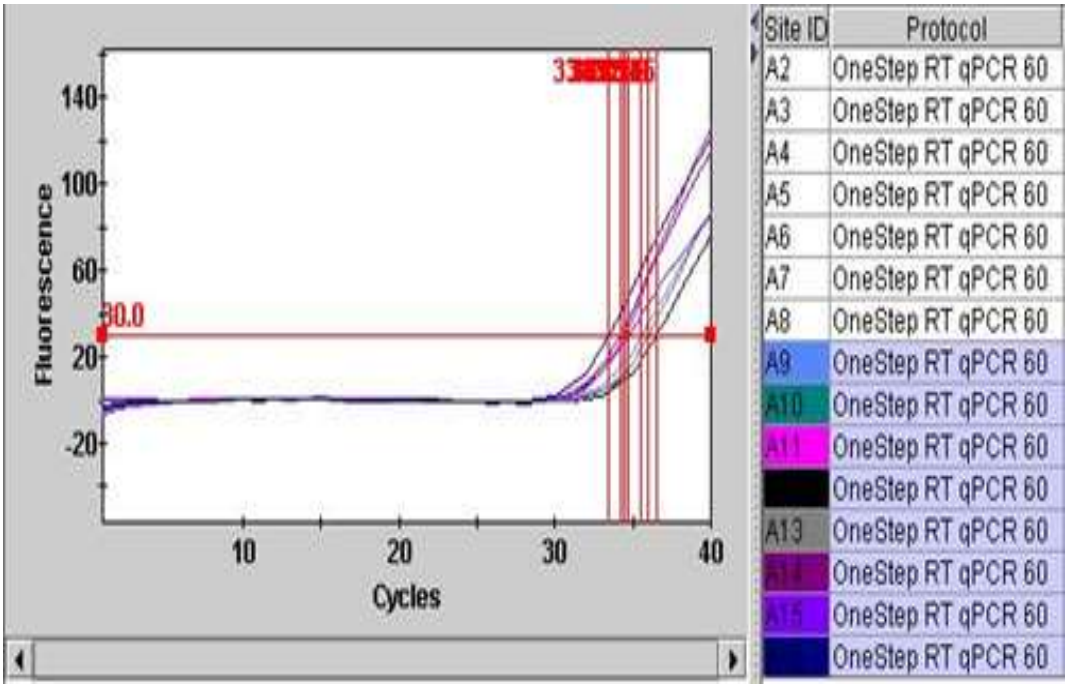

Figure 4-Ct values of *msrB*genein *S.aureus* isolates (SA3, SA13,SA32,SA10) (A9:SA3 , A11:SA13, A13:SA32 , A15:SA10): calibrator , (A10:SA3 ,A12:SA13 , A14 :SA32 , A16:SA10): with antibiotic (clarithromycin) .

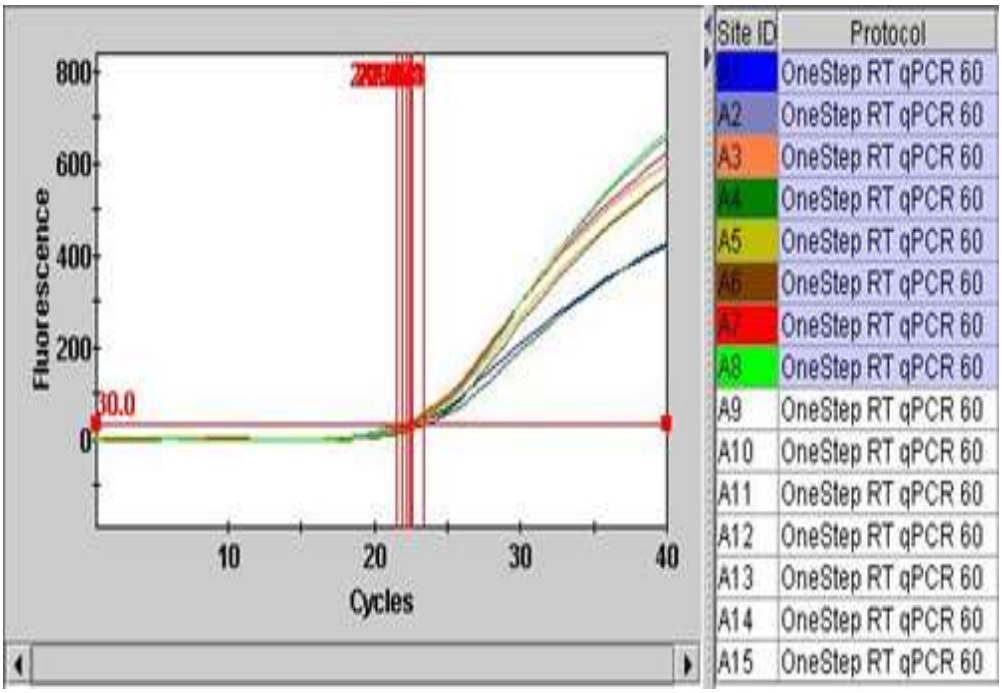

Figure 5-Ct value of *tpi*genein *S.aureus* isolates (SA3, SA13,SA32,SA10), (A1:SA3 , A3:SA13, A5:SA32 , A7:SA10): calibrator , (A2:SA3 ,A4:SA13 , A6 :SA32 , A8:SA10): with antibiotic (clarithromycin). .

Table 8-Fold changes in expression of *msrB*after treated with clarithromycin

	Calibrator			**Clarithromycin**				
Sample	**Ct*msrB* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**Ct*msrB* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**ΔΔCt**	**Fold change**
SA40	33.1	17.1	16	33.2	23.2	10	-6	64
SA32	38.8	23.1	15.7	36.7	23.2	13.5	-2.2	4.6
SA13	33.8	20.6	13.2	33.4	22.9	10.5	-2.7	6.5
SA30	32.9	17.1	15.8	34.7	19.2	15.5	-0.3	1.2
SA10	34.7	22.2	12.5	33.5	22.6	10.9	-1.6	3
SA36	35.3	18.8	16.5	33.2	19.3	13.9	-2.6	6
SA3	35.5	22.5	13	35.9	21.6	14.3	1.3	0.4
SA7	39.7	16.4	23.3	33.9	15.4	18.5	-4.8	27.9

Effect of clindamycin onexpression of *linA/linA'* gene

To determine the effect of clindamycin on the expression of *linA/linA'* gene in *S.aureus* isolates, qRT-PCR was performed to quantify *linA/linA'* mRNAexpression in isolates. The results showed that there were induction in the expression of this gene in isolates SA13 and SA30 at concentration below MIC value (4µg/ml)(fold changes were 1.5 and 48.5 respectively).Since these two isolates had cMLS resistance phenotype for lincosamide (clindamycin) which mean thatthe expression had been carried

out so the resistance was due to *linA/linA'* gene and theresistance occured for these isolates ,nucleotidyltransferase encoded by *linA/linA'* gene causedenzymatic inactivation of clindamycin through nucleotidylation [34-35].The rest isolates (iMLS , M and SAB resistance phenotypes) showed reduction in *linA/linA'*expression atconcentration below MIC value (0.1 µg/ml)because these isolates had susceptibility to clindamycin so this antibiotic supressed the expression of *linA/linA'* gene as shown in Figures-(6, 7)and Table-9, the reduction in *linA/linA'* gene expression in isolates SA40 and SA32 (iMLS) indicated that *S.aureus* isolates remain sensitive to clindamycin due to the abcense of inducer (macrolides) antibiotics which led to induce lincosamide resistance [36].The inhibitory effect of clindamycin is due to effecient membrane penetrating ability, resulting in a higher intracellular concentration [37].

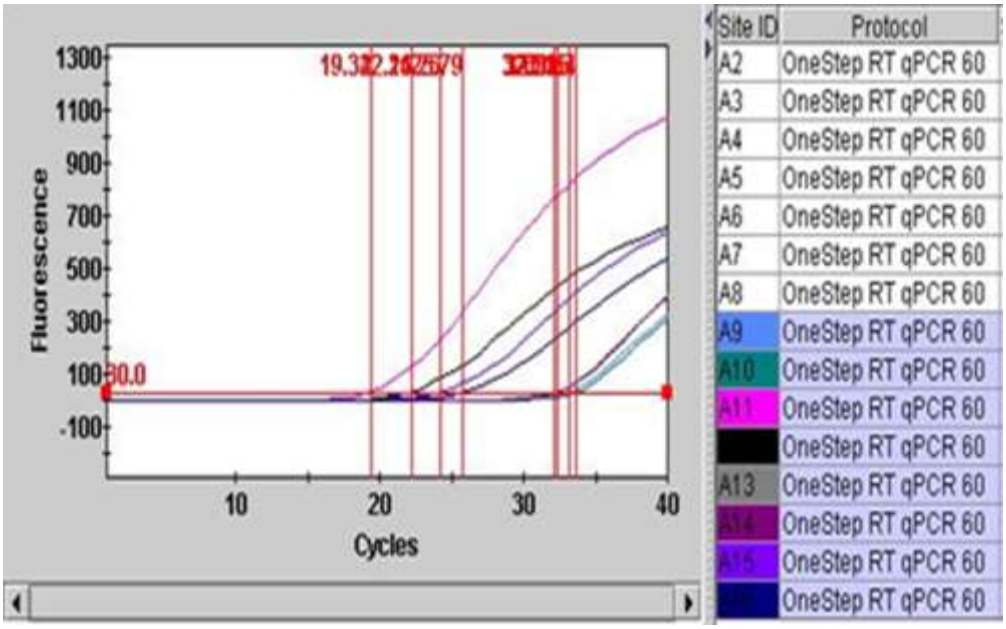

Figure 6-Ct value of *linA/linA'*genein *S.aureus* isolates (SA30, SA7,SA40,SA10) **,**(A9:SA30 , A11:SA7, A13:SA40 , A15:SA10): calibrator , (A10:SA30 ,A12:SA7 , A14 :SA40 , A16:SA10): with antibiotic(clindamycin).

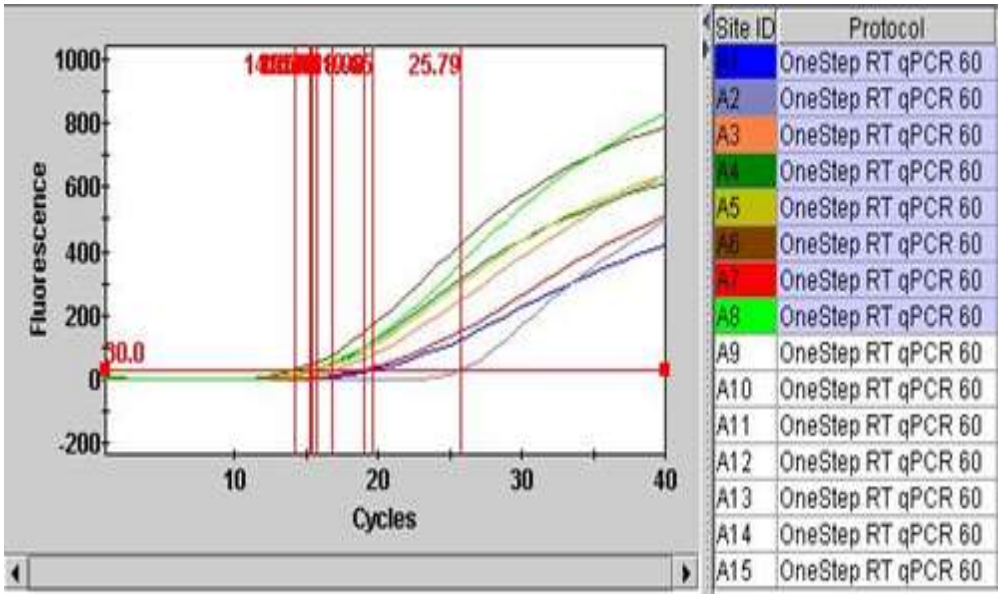

Figure 7-Ct value of *tpi*genein *S.aureus* isolates (SA30, SA7,SA40,SA10),, (A1:SA30 , A3:SA7, A5:SA40 , A7:SA10): calibrator , (A2:SA30 ,A4:SA7 , A6 :SA40 , A8:SA10): with antibiotic (clindamycin).

Table 9-Fold changes in expression of *linA/linA'* after treated with clindamycin

	Calibrator			Clindamycin				
Sample	**Ct *linA/linA'* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**Ct *linA/linA'* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**ΔΔCt**	**Fold change**
SA40	32.1	15.7	16.4	32.2	14.2	18.1	1.7	0.3078
SA32	30.7	17.1	13.6	32.2	17.1	15.1	1.5	0.4
SA13	32.20	17.1	15.1	33.1	18.6	14.5	-0.6	1.5
SA30	33.1	19.6	13.5	33.6	25.7	7.9	-5.6	48.502
SA10	24.2	18.9	5.3	25.7	15.3	10.4	5.1	0.0292
SA36	32.3	18.8	13.5	32.6	15.8	16.8	3.3	0.1
SA7	19.3	16.8	2.5	22.1	15.3	6.8	4.3	0.0508
SA3	27.2	20.6	6.6	33.2	18.6	14.6	8	0.004

Effect of lincomycin on expression of *linA/linA'* gene

In order to determine the effect of lincomycin on the expression of *linA/linA'* gene in *S.aureus* isolates after 18 hrs.of exposure time, qRT-PCR was used to quantify *linA/linA'* mRNAexpression in isolates. The results showed that there was induction in the expression of this gene in isolate SA13 which had cMLS resistance phenotype atconcentration below MIC value (4µg/ml). This mean that lincomycin play an important role as an inducer of resistance gene expression,resistancetolincosamide occurred by the presence of *linA/linA'* gene which act as an O-nucleotidyltransferase which inactivated linosamides [38].The rest isolates showed reduction in expression atconcentration below MIC value (0.1 µg/ml), solincomycin had an inducer activity of the dissemination of resistance determinant [39].This mean that the effect of antibiotic in inhibition of mRNA transcription and expression of *linA/linA'* gene [40].Figures-(8, 9) and Table-10.

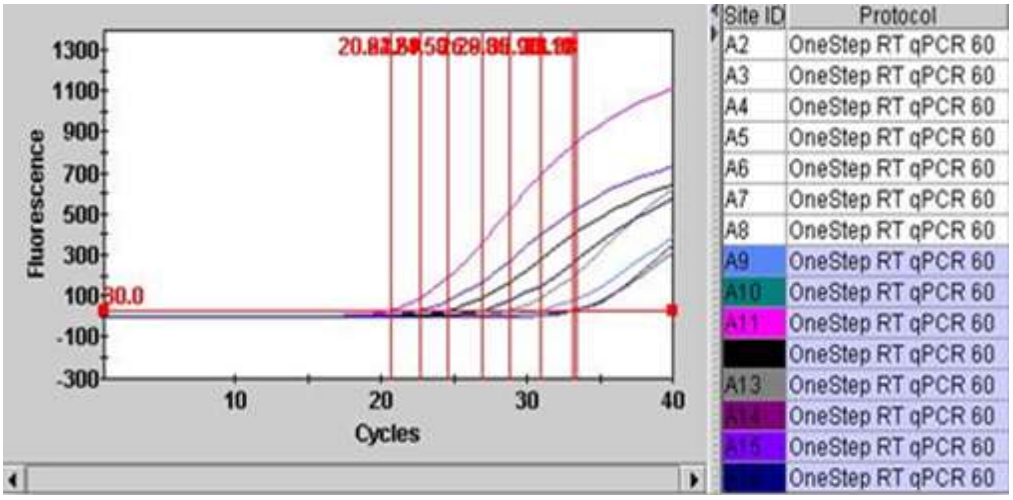

Figure 8-Ct value of *linA/linA'*genein *S.aureus* isolates (SA3, SA7,SA30,SA10) (A9:SA3 , A11:SA7, A13:SA30 , A15:SA10): calibrator , (A10:SA3 ,A12:SA7 , A14 :SA30 , A16:SA10): with antibiotic (lincomycin) .

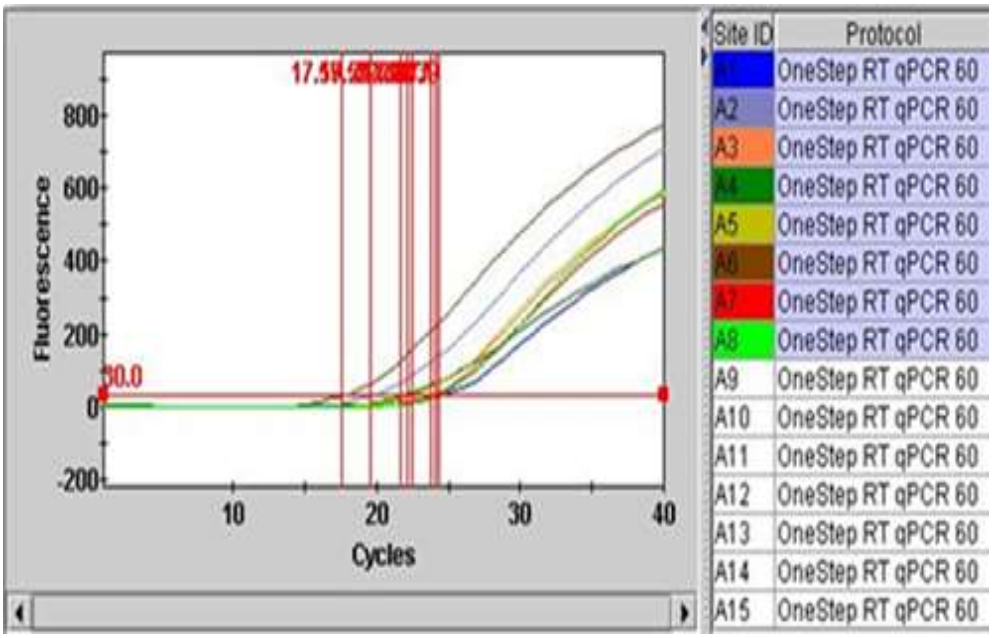

Figure 9-Ct value of *tpi*genein *S.aureus* isolates (SA3, SA7,SA30,SA10) , (A1:SA3 , A3:SA7, A5:SA30 , A7:SA10): calibrator (A2:SA3 ,A4:SA7 , A6 :SA30 , A8:SA10): with antibiotic (lincomycin)**.**

Table 10-Fold changes in expression of *linA/linA'* after treated with lincomycin

	Calibrator			**Lincomycin**				
Sample	**Ct *linA/linA'* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**Ct *linA/linA'* (mean)**	**Ct*tpi* (mean)**	**ΔCt**	**ΔΔCt**	**Fold change**
SA40	27.2	20.6	6.6	30.5	18.6	11.9	5.3	0.025
SA32	30.7	17.1	13.6	31.2	17.1	14.1	0.5	0.7
SA13	32.2	17.1	15.1	29.3	18.6	10.7	-4.4	21.11
SA30	28.9	22.5	6.4	33.4	17.6	15.8	9.4	0.0015
SA10	22.7	23.8	-1.1	26.9	24.1	2.8	3.9	0.0670
SA36	32.2	18.8	13.4	32.1	15.8	16.3	2.9	0.14
SA3	31	24.4	6.6	33.2	19.6	13.6	7	0.0078
SA7	20.6	22.2	-1.6	24.6	21.7	2.9	4.5	0.0442

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