Egypt. J. Microbiol. **Vol. 55,** pp. 13-19 (2020)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

Detection of Clindamycin Resistance Antibiotic Genes among *Staphylococcus* Isolates by Using Real Time PCR

Mahmoud Abd El-Mongy[(1)#], Amira El-bokl[(1)], Amira El-Hendy[(2)]
[(1)]Microbial Biotechnology Department. Genetic Engineering & Biotechnology Research Institute. University of Sadat City Egypt; [(2)]Clinical Pathology Department, Faculty of Medicine, Minoufiya University, Shibin El-Kom, Egypt.

STAPHYLOCOCCUS aureus is the most common pathogens and an important nosocomial pathogen. This bacterium has ability to acquire resistance to different antibiotic classes. Erythromycin and clindamycin have been used for treating skin and soft-tissue infections caused by these bacteria. Although, expression of macrolide-lincosamide-streptogramin B resistance (MLSB) can limit the effect of these drugs.

This study aimed to detect the presence of genes encoding resistance to macrolides, lincosamides and streptogramins (MLSB) among clinical *Staphylococcus* strains.

A total of 100 *Staphylococcus* isolates from clinical specimens (blood, sputum and wound) were collected. All these isolates were identified biochemically. They were tested against different antibiotics and double-disk diffusion method and genes were detected using PCR.

Results show that 63 isolates were genotypically tested by using real time PCR for detection of Erm (B) and Erm (C) genes. (EryS ClinS) was detected in 50 (50%) of the isolates followed by constitutive phenotype of MLSB resistance (EryR ClinR) 29 (29%) and inducible MLSB resistance (EryRClinInd) 17 (17%), while the MSB phenotype (EryR ClinS) 4(4%) was the least frequent. 51(80.9) isolates have Erm (B) gene positive 33(52.3) strains have Erm (C) gene positive.

This study investigate that the double disk diffusion test is a suitable test for detection of staphylococci which are resistant to clindamycin and should be used as fast detection and help in treatment of patients. And thus, the screening procedure using Real time PCR is sensitive in detection of Erm genes which are responsible for inducible resistance to clindamycin.

Keywords: Erm genes, Inducible clindamycin resistance, Real Time PCR, Staphylococci.

Introduction

Staphylococcus aureus is one of the most frequent pathogenic bacteria which cause diseases to human (Al-Ruaily & Khalil, 2011). Staphylococcus aureus is a Gram-positive bacterium and causes many infectious diseases to human such as bacteremia, skin infections, endocarditis, pneumonia and food poisoning. However, increasing use of MLSB antibiotics increases in the number of Staphylococcal strains acquiring resistance to MLSB antibiotics (Deotale et al., 2010). Detecting of MRSA isolates was done by a mobile genetic element mec A and using polymerase chain reaction PCR. This technique characterized with (93.8 to 100%) sensitivity and (98.6 to 100) specificity (Ratnayake & Olver, 2011). Resistance mechanism of Macrolides against different types of bacteria especially Gram-positive cocci

[#]Corresponding author email: abdelmongyd@yahoo.com
Received 16/12/2019; Accepted 24/2/2020
DOI: 10.21608/ejm.2020.19869.1133

recorded by Erm genes and can occur in two mechanisms: an active efflux mechanism and Erm genes encode enzymes that confer inducible resistance to macrolides agent via methylation of the 23S rRNA (Pal et al., 2010; Khashei et al., 2018).

Clindamycin is an antibiotic used against various types of bacterial infections such as pneumonia, joint and other diseases caused to human by staphylococcus aureus (Mukesh et al., 2006). In this work, we used real time PCR for detection of Erm (B) and Erm (C) genes in clinical *Staphylococcus* isolates, which is responsible for the inducible resistance to clindamycin and demonstrates the applicability of this technique in detecting these genes in the isolates.

In fact macrolides lincosamides and streptogramin B all of them have same target site which is protein biosynthesis, erythromycin belong to macrolides and can induce cross-resistance against two other groups, So *S. aureus* isolates which show resistance to erythromycin will resist to linocosamide and strptogramin B (Stefanie & Gallert, 2014).

Materials and Methods

Specimen collection

This study was performed in the Clinical Pathology Department, Faculty of Medicine, Minoufiya University. Specimens from blood, sputum, and wound were aseptically collected and transported immediately to the microbiology laboratory.

Identification of isolates

S. aureus and CoNS were identified by using Gram stain and Biochemical tests (Catalase Test, Coagulase Test and DNase test)**.**

Susceptibility tests

The isolates were tested for susceptibility to clindamycin and erythromycin oxacillin, azithromycin, amoxycillin, -clavulanic acid, vancomycin and ciprofloxacin, cefoxitin, ampicillin, methicillin, cefamandole amoxyclav, aztreonam, amoxycillin. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates that were erythromycin-resistant (ER-R) and clindamycin-sensitive (CL-S) were tested for inducible resistance by the D-test. Erythromycin and clindamycin discs were placed 15 mm apart (edge to edge) on Mueller Hinton agar plate (Fig. 1). Following incubation at 37°C for 24hrs, D-test positivity (ICR) was identified by flattening of the clindamycin zone between the erythromycin and clindamycin discs. The D-test was considered negative in the absence of flattening of the clindamycin zone.

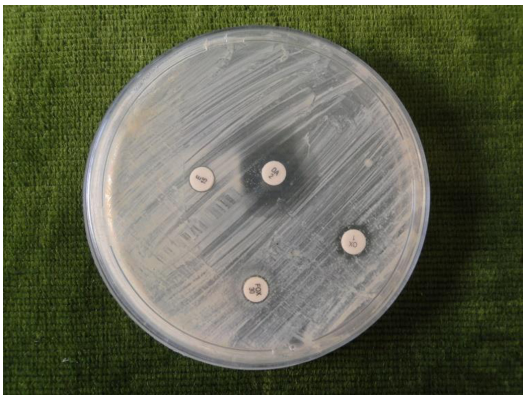

Fig.1. D- Test showing blunting of zone of inhibition around Clindamycin towards Erythromycin disc indicating iMLSB phenotype.

Detection of Erm (B) and Erm (C) genes

Genomic DNA was obtained by using GeneJET Genomic DNA Purification Kit Protocol. The extracted DNA was used for detection of Erm genes by using real time PCR *Erm* (B) and *Erm* (C) genes were detected by real time PCR using the oligonucleotide primer pairs described previously (Díaz et al., 2007) (Table 1). These oligonucleotides were synthesized by South McDowellBlvd, Petaluma –USA.

TABLE 1. Primers used for the detection of Erm genes.

Target gene	Target sequence of the primers	Size of amplified fragment (bp)
Erm (B)	5'-CTA TCT GAT TGT TGA AGA AGG ATT-3' 5'-GTT TAC TCT TGG TTT AGG ATG AAA-3'	141
Erm (C)	5'-CTT GTT GAT CAC GAT AAT TTC C-3' 5'-ATC TTT TAG CAA ACC CGT ATT C-3'	189

All solutions were gently vortexed and briefly centrifuged after thawing. The master mix was prepared by adding the 10µL master mix, 0.2µL primer and 4.8µL water for each qPCR reaction to a tube at room temperature. The master mix was mixed thoroughly and 15µL of the reaction was dispensed into PCR plates. 5µL of template DNA (≤ 200ng/ reaction) was added to the individual PCR plates containing the master mix. The thermal cycler was programed according to the recommendations below and the samples were placed in the cycler and the program was started. The PCR mixture was subjected to thermal cycle, (1 cycle of 2min at 50°C as UDG pre-treatment step, 10min at 95°C for 1 cycle as the initial denaturation step, 15sec at 95°C for 40 cycles as denaturation step, 30sec at 60°C for 40 cycles as an annealing step and of 30sec at 72°C for 40 cycles as the extension step, Data acquisition was performed during the extension step.

Results

From 100 Staphylococcal samples, MRSA was detected (45%), MSSA (8%), MRCoNS, (13%) and MSCoNS 34/100 (34%). Table 2 shows that there was highly statistically significant relation between positivity of Erm genes and presence of clindamycin resistance and 100% of ERY-S, CL-S was Erm (b & c) negative. Antibiotic susceptibility patterns for our isolates were detected as the following: clindamycin resistance 53% from the total number of isolates. Erythromycin resistance 48%, Efoxitin resistance 57% Oxacillin resistance 57%, methicillin resistance 77%, ampicillin resistance 46%, ciprofloxacilin resistance was 62%, cefamandole résistance 83%, amoxyclav 40%, vancomycin 80, aztreonam resistance was 86% amoxycillin resistance was 77% and azithromycin resistance was 77%. The pattern of (EryS ClinS) was detected in 50(50%) of isolates, followed by constitutive phenotype of MLSB resistance (EryR ClinR) 29 (29%), and inducible MLSB resistance (EryR ClinInd) 17 (17%), while the MSB phenotype (EryR ClinS) 4(4%) was the least frequent (Table 3).

Only 63 strains were genotypically tested for genes Erm (B) and Erm (C). The Erm (B) gene was detected in 51 isolates (28 *S. aureus*)18 isolates of them were phenotypically constitutive MLSB,10 were inducible MLSB, but MSB phenotype not detected, also Erm (B) gene was detected in 21 isolates which were CNS10 isolates of them were phenotypically constitutiveMLSB,7 isolates were inducible MLSB, and only 3 isolates were MSB phenotype, the Erm (C) gene was found in 33 isolates (15 S. *aureus*) 5 isolates of them were phenotypically constitutive MLSB, 10 were inducible MLSB, but MSB phenotype not detected, also Erm (C) gene was detected in 18 isolates which were CNS11 isolates of them were phenotypically constitutive MLSB,7 isolates were inducible MLSB, but MSB phenotype not detected (Fig. 2 and 3).

TABLE 2. Relationship between phenotypes and Erm genotypes among the studied cases .

	Clindamycin Resistance										Test	P value
	ERY-S,CL-S N = 11		ERY-R,CL-R (Constitutive MLS) N = 29		ERY-R,CL-S (D Test positive iMLS)N=17		ERY-R,CL-S (D Test negative MSB)N= 4		EryS ClinR N = 2			
	No	%	No	%	No	%	No	%	No	%	X^2	
Erm (B) Positive	0	0	29	100	17	100	3	75	2	100	58.1	<0.001
Negative	11	100	0	0	0	0	1	25	0	0		
Erm (c) Positive	0	0	16	55.2	17	100	0	0	0	0	34.2	<0.001
Negative	11	100	13	44.8	0	0	4	100	2	100		
Combined erm(B)/erm(C) erm (B)	0	0	13	55.2	0	0	3	75	2	100		
Combined erm((B & C)	0	0	16	44.8	17	100	0	0	0	0	80.6	<0.001
Non erm (B& C)	11	100	0	0.0	0	0	1	25	0	0		

TABLE 3. Correlation between Erm genes and MLSB resistance phenotype.

Isolates	Clindamycin resistance	Erm genes				X^2	P value
		Erm (b)		Erm (c)			
		No	%	No	%		
Staph	ERY-S,CL-S	0	0.0	0	0.0	30.0	<0.001
	ERY-R,CL-R (Constitutive MLS)	18	62.1	5	31.3		
	ERY-R,CL-S (D test positive iMLS)	11	37.9	11	68.7		
	Total	29	100	16	100		
CNS	ERY-S,CL-S	0	0.0	0	0.0	29.6	<0.001
	ERY-R,CL-R (Constitutive MLS)	11	50.0	11	64.7		
	ERY-R,CL-S (D test positive iMLS)	6	27.3	6	35.3		
	ERY-R,CL-S (D test negative MSB)	3	13.6	0	0.0		
	EryS ClinR	2	9.1	0	0.0		
	Total	22	100	17	100		

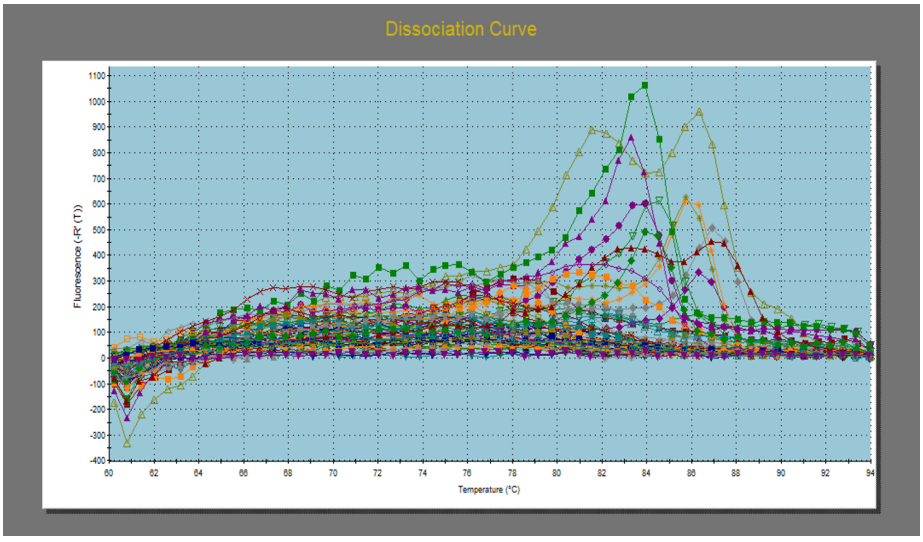

Fig. 2. The dissociation curve of Erm (B) gene.

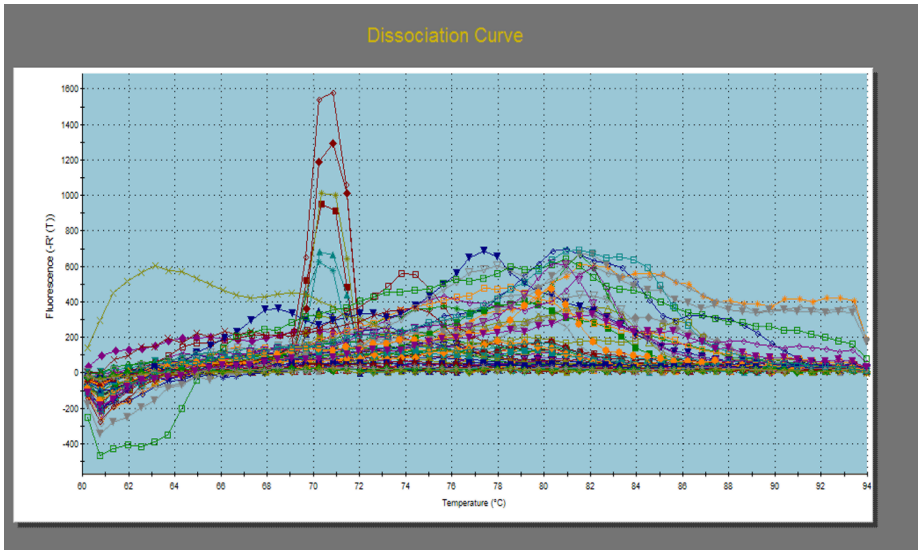

Fig. 3. The dissociation curve of Erm (C) gene.

In this study, we found that the pattern of (EryS ClinS) was detected in 50 (50%), followed by constitutive phenotype of MLSB resistance (EryR ClinR) 29 (29%), and inducible MLSB resistance (EryRClinInd) 17 (17%), while the MSB phenotype (EryR ClinS) 4(4%) was the least frequent. This result was in agreement with Coutinho et al. (2010) as their study showed that total of 46.7% of staphylococci was positive for cMLSB; 3.3% for iMLSB and 3.3% for MSB. On the other hand, this result disagrees with Pal et al. (2010) as their study showed that constitutive resistance was demonstrated in (46.97%), inducible clindamycin resistance in (23.48%) and MS (29.53%) and also disagree with Deotale et al. (2010) as their study showed that 36 (14.5%) isolates showed inducible resistance to clindamycin, 9(3.6%) gave constitutive resistance while other strains 35 (14.1%) showed MS phenotype.

Antibiotic susceptibility patterns for our isolates was in agreement with a previous study (Lyall et al., 2013) as their study showed that all the isolates were sensitive to vancomycin. This result was in agreement with Pal et al. (2010). Sensetivity of iMLSB phenotype isolated were ampicillin 37.5% amoxyclav 39.13%, ciprofloxacin 78.78%, and vancomycin 100%.

In this study, only 63 strains were genotypically tested for genes Erm (B) and Erm (C). The Erm (B) gene was founded in 51 isolates 18 isolates of them were phenotypically. Constitutive MLSB, 10 were inducible MLSB , but MSB phenotype not detected, also Erm (B) gene was detected in 21isolates which were CNS10 isolates of them were phenotypically constitutive MLSB, 7 isolates were inducible MLSB, and only 3 isolates were MSB phenotype, the Erm (C) gene was found in 33 isolates (15 S. aureus) 5 isolates of them were phenotypically constitutive MLSB,10 were inducible MLSB, but MSB phenotype not detected, also Erm (C) gene was detected in 18 isolates which CNS11 isolates of them were phenotypically constitutive MLSB,7 isolates were inducible MLSB, but MSB phenotype not detected.

This result was in agreement with Shahsavan et al. (2012) as their study showed that Erm (C) was found in (72%) of isolates and Erm (B) was detected in (69%) of isolates, Erm (C) was more common in the isolates with the constitutive phenotype. This result was in agreement with Coutinho et al. (2010) as their study showed that one or more Erm genes were present in 50.1% of isolates. Erm (C) was detected in 29 isolates and Erm B in 3 isolates. This result was in agreement with Cetin et al. (2010) as their study showed that the Erm (C) gene was found as a single MLSB resistance gene in eight (17%) *S. aureus* isolates Erm B gene was present only in three MRCoNS strains with the cMLSB resistance phenotype and one of these strains also contained Erm (C).

Conculusion

Real time PCR should be used for Erm A gene detection as it showed a great correlation with D-test results finally; use of double–desk test is very important to detect clindamycin resistant *staphylococci* and real time PCR also used to fast detection of genes responsible for resistance of antibiotics to facilitate treatment.

Acknowledgement: The corresponding author would like to thank all scientists who have assisted in this manuscript. We must thank especially member of Microbial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City for helpful support and advice.

Reference

Al- mussawi, A.A. (2014) Detection of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) from human clinical specimens using conventional biochemical tests and cromogenic media. *Indian Journal of Applied Research,* **4**(2), 249-555.

Al-Ruaily, M.A., Khalil, O.M. (2011) Detection of (mecA) gene in methicillin resistant *Staphylococcus aureus* (MRSA) at Prince A/ Rhman Sidery Hospital, Al-Jouf, Saudi Arabia. *Journal of Medical Genetics and Genomics,* **3**(3), 41-45.

Cetin, S.E., Gunes, H., Kaya, S., Aridogan, C.B., Demirci, M. (2010) Distribution of genes encoding resistance to Macrolides, Lincosamides and Streptogramins among clinical Staphylococcal isolates in a Turkish University Hospital. *J. Microbiol. Immunol. Infect*. **43**(6), 524-529.

Coutinho, V., Paiva, M.R., Reiter, C.K., Paris, F., Barth, L.A., Mombach, B.A., Machado, P. (2010) Distribution of erm genes and low prevalence of inducible resistance to clindamycin among

Staphylococci isolates. *Braz. J. Infect. Dis.* **14**(6), 564-568.

Deotale, V., Mendiratta, D.K., Raut, U., Narang, P. (2010) Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. *Indian Journal of Medical Microbiology,* **28**(2), 124-126.

Díaz, M.L., Casa, C.A., Sánchez, T.M., Martín, A.J. (2007) Detection of inducible resistance to clindamycin in cutaneous *Staphylococcus* spp. by phenotypic and genotypic methods. *Nurse Clin. Microbiol.* **25**(2), 77-81.

Khashei, R., Malekzadegan, Y., Hadi Sedigh Ebrahim-Saraie, Razavi, Z. (2018) Phenotypic and genotypic characterization of macrolide, lincosamide and streptogramin B resistance among clinical isolates of staphylococci in southwest of Iran. *BMC Res Notes*, **11**, 711.

Lyall, K.D., Gupta, V., Chhina, D. (2013) Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus. Journal of Mahatma Gandhi Institute of Medical Sciences*, **18**(2), 112-115.

Lozano, C., Aspiroz, C., Lasarte, J.J., Gómez-Sanz, E., Zarazaga, M., Torres, C. (2011) Dynamic of nasal colonization by methicillin-resistant *Staphylococcus aureus* ST398 and ST1 after mupirocin treatment in a family in close contact with pigs. Comp. *Immun. Microbiol. Infect. Dis.* **34**, 1-7.

Mukesh, P., Ken, W.B., Stephen, M.A., Gretchen, C., Craig, H. (2006) Prevalence of inducible clindamycin resistance among Community and hospital associated *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* **44**(7), 2481-4.

Pal, N., Sharma, B., Sharma, R., Vyas, L. (2010) Detection of Inducible Clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India. *J. Postgrad Med.* **56**(3), 182-185.

Ratnayake, L., Olver, W.J. (2011) Rapid PCR detection of methicillin resistant *Staphylococcus aureus* and methicillin-sensitive *S. aureus* samples from charcoal-containing blood culture bottles. *J. Clin. Microbiol.* **49**(6), 2382.

Shahsavan, S., Emaneini, M.; Khoshgnab, N.B., Khoramian, B., Taherikalani, M.A. (2012) High prevalence of mupirocin and macrolide resistance determinant among *Staphylococcus aureus* strains isolated from burnt patients. *Burns*, **38**(3), 378-382.

Stefanie, Heß, Gallert, C. (2014) Resistance behaviour of inducible clindamycin-resistant staphylococci from clinical samples and aquatic environments. *Journal of Medical Microbiology*, **36**, 11.

الكشف عن جينات المضادات الحيوية المقاومة للكليندامايسين بين عزلات المكورات العنقودية باستخدام تفاعل البلمرة المتسلسل الحقيقي

محمود عبدالمنجى[1]، اميرة البكل[1]، اميرة الهندى[2]

[1]معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية - جامعة مدينة السادات – مصر، [2]كلية الطب جامعة المنوفية - شبين الكوم- مصر.

المكورات العنقودية هي واحدة من مسببات الأمراض الأكثر شيوعا المعزولة من الالتهابات والأمراض المكتسبة من المجتمع. كانت المضادات الحيوية مثل الإريثروميسين والكليندامايسين خيارات مفيدة لعلاج التهابات الجلد والأنسجة الرخوة التي تسببها المكورات العنقودية. ومع ذلك، يمكن للتعبير عن مقاومة الماكروليد لينكوساميد - ستربتوجرامين ب (MLSB) أن تحد من فعالية هذه الأدوية. تهدف دراستنا إلى التحقق من وجود الجينات التي ترمز لمقاومة الماكروليدات واللينكوساميد والستربتوجرامات (MLSB) بين عزلات المكورات العنقودية السريرية. تم جمع ما مجموعه 100 من المكورات العنقودية المعزولة من العينات السريرية (الدم والبلغم والجرح). تم تعريف كل هذه العزلات بيوكيميائيا. تعرضوا لاختبار الحساسية المضادة للميكروبات وطريقة نشر القرص المزدوج واكتشف الجين باستخدام Real Time PCR. تم اختبار 63 عزلة وراثيا باستخدام PCR في الوقت الحقيقي للكشف عن الجينات (B) Erm و (C) Erm. تم الكشف عن (EryS ClinS) في 50 (50%) من العزلات تليها النمط الظاهري التأسيسية لمقاومة MLSB 29 (EryR ClinR) (29%) ومقاومة MLSB المستحثة (17%) 17 (EryRClinInd) ، في حين أن النمط الظاهري MSB (كان 4) 4 (EryR ClinS) الأقل تواترا. 51 (80.9) عزلة لها سلالات إرم (B) إيجابية الجين 33 (52.3) سلالات لديها جين ERM C إيجابي. أوضحت هذه الدراسة أنه يجب استخدام اختبار نشر القرص المزدوج كوسيلة إلزامية في اختبار نشر القرص الروتيني للكشف عن مقاومة الكليندامايسين المستحثة في المكورات العنقودية من أجل العلاج الأمثل للمرضى. وبالتالي ، فإن إجراء الفحص باستخدام Real time PCR حساس في الكشف عن جينات Erm المسؤولة عن المقاومة الحثية للكليندامايسين.