

MOLECULAR DETECTION OF *erm(A)*, *mef(A)* IN *Staphylococcus* spp AND *Streptococcus* spp RESISTANT TO MACROLIDE FROM DIFFERENT CLINICAL INFECTIONS

Hadi R. Rasheed Al-Taai

Dept. of Biology-College of Sciences-University of Diyala, Iraq (drhadialtaai@yahoo.com)

ABSTRACT

Isolates *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus albus*, *Streptococcus Pyogenes* and *Streptococcus agalactiae* isolated from different clinical infections were activated. Results of susceptibility test showed that *Staphylococcus aureus* have resistance to antibiotics of erythromycin, azithromycin and clindomycin with percentage 45%, 45% 25% respectively, while *Staphylococcus epidermidis* resistant to erythromycin, azithromycin and clindomycin with ratio 66.6%, 44%, 22.2%, respectively. The resistance of erythromycin and azithromycin by *Streptococcus pyogenes* reached 25% and 37.5% respectively, whereas all isolates sensitive to clindomycin. The outcome disclosed that the values of minimum inhibitory concentrations of erythromycin ranged between 32 - \geq 64 μ g/ mL for all isolates. The results outcomes to 13(65%) isolates of *Staphylococcus aureus* produced Staphylokinase, 6(66.6%) isolates of *Staphylococcus epidermidis* able to produce the same enzyme. The results proved that 3(37.5%) isolates of *Streptococcus pyogenes* able to produce Streptokinase enzyme, but *Streptococcus agalactiae* could not produce streptokinase. The PCR-based assay, gene was detected in *erm (A)* gene was detected in 33.3% isolate of *Staphylococcus spp.* and 50% of *Streptococcus spp.*, whereas *mef (A)* was detected in 46.6 % isolates of *Staphylococcus spp.* and 50% of *Streptococcus spp.* These findings demonstrated the variable phenotypic expression when compared to genotypic detection .

Key words: *Staphylococcus* spp., *Streptococcus* spp, Macrolide, *erm(A)*, *mef(A)*. PCR assay.

INTRODUCTION

The *Staphylococci* are a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteraemia. In spite of large-scale efforts to control their spread, they persist as a major cause of both hospital and community acquired infections worldwide. The two major opportunistic pathogens of this genus are *Staphylococcus aureus* and *Staphylococcus epidermidis* (Fung-Tomc *et al.*, 2002 ; Gill *et al.*, 2005). Unlike coagulase-negative *Staphylococci* (CNS), *Staphylococcus aureus* strains are able

to secrete free plasma coagulase, which is an important virulence factor for these bacteria. *S. aureus* is a common pathogen in nosocomial infections, so exact identification of *S. aureus* isolates is essential for microbiology laboratories (Emori & Gaynes, 1993). The proportion of clinical *S. aureus* isolates that are resistant to methicillin (MRSA) has increased over recent years (Tiemersma *et al.*, 2004). Compared with methicillin-sensitive *S. aureus* (MSSA), infections by MRSA strains are associated with increased morbidity and mortality in affected patients (Cosgrove *et al.*, 2003). Macrolide resistance in *Streptococcus pyogenes* results primarily from modification of the drug target site by methyl transferase encoded by *erm* genes, *erm(A)* and *erm(B)* or by active efflux mediated by a *mef*-encoded efflux pump. Of these, *erm(A)* is inducibly expressed (Malhotra-Kumar *et al.*, 2009) and generally confers low-level resistance to macrolides, whereas lincosamides and streptogramins B (MLSB), which share overlapping binding sites, remain active against *erm(A)*-harboring *S. pyogenes* (Leclercq, 2002). *erm(B)*-mediated erythromycin resistance is the most common mechanism in many areas of the world, including some European and Asian countries (Monaco *et al.*, 2005 ; Reinert *et al.*, 2005), whereas *mef*, with its most common variants *mef(A)* and *mef(E)*, is predominant in the United States (Farrell *et al.*, 2007), Canada (Hoban *et al.*, 2001), and the United Kingdom (Amezaga *et al.*, 2002). That macrolide used is the main driver of macrolide resistance in streptococci has been well demonstrated at the population and individual levels (Goossens *et al.*, 2005 ; Malhotra-Kumar *et al.*, 2007). Because *erm* and *mef* are cocarried with *tet* genes on mobile elements, tetracycline also use affects macrolide resistance (Malhotra-Kumar *et al.*, 2007). In Iraq such molecular studies about these important genes are rare so this study was done to focus on detection of *erm A* and *mef A* genes present in *Staphylococcus* spp. and *Streptococcus* spp. using phenotypic test followed by genotypic using specific primers for these target gene by PCR technique .

MATERIAL AND METHODS

Activation of isolates

Forty isolates of *Streptococcus* spp and *Staphylococcus* spp were isolated from 200 samples clinical sources included (Urine, blood, middle ear, sputum, throat, wounds, and vaginal swabs) from hospitals (Baquba teaching hospital, Al-Batool hospital and Balad Ruz teaching hospital) in addition to some health centers for the period 1/9/2013 until 01/01/2014. Blood agar base medium, mannitol salts were used for activation isolates of *Streptococcus* spp and *Staphylococcus* spp., Nonobiocin disc for discrimination *S. saprophyticus*, while

bacitracin disc and optochin disc were used for discrimination *Streptococcus*. Identification of all bacterial isolates was confirmed mediated Vitek2 system.

Susceptibility Testing

Susceptibility of all bacterial isolates to azithromycin, erythromycin and clindamycin antibiotics was determined by the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012). Each antibiotic concentration was applied on the surface of Muller-Hinton agar plates inoculated with *S. epidermidis*, *S. aureus*, *Strp. pyogens*, *Strp. agalactiae*, *S. saprophyticus* isolates and incubated at 37 °C for 24 h. (Tankhiwale *et al.*, 2004).

Minimum inhibitory concentration (MIC)

MIC was determined by agar dilution method in Muller Hinton agar (Oxoid/ UK). Serial two-fold dilution of erythromycin was incorporated in media plates from 0.25 to 1024 µg ml⁻¹ as recommended by Clinical and Laboratory Standards Institute (CLSI, 2012).

Staphylokinase and Streptokinase Production

The Staphylokinase and Streptokinase Production of the different bacterial isolates were assayed as described by (Cruickshank *et al.*, 1975; Rajamohan *et al.*, 2000).

PCR amplification

Isolates with positive phenotypic tests were subjected to molecular screening study using PCR amplification technique according to following steps:

Extraction of bacterial DNA

Bacterial DNA was isolated using a DNA isolation kit (Geneaid Biotech. Ltd; Taiwan)

PCR amplification procedure:

Detection of *erm A*, *mef A* genes was performed by amplifying the genes by PCR. The primers sequences were previously reported by (Sutcliffe *et al.*, 1996a ; Sutcliffe *et al.*, 1996 b ; Lim, 2002) and obtained from Korea/Bioneer. Descriptions and sequences of the PCR primers used in this study are displayed in Table 1. Amplification was performed in a thermal cycler (Eppendorf, Germany) according to the methods described by (Sutcliffe *et al.*, 1996 a ; Sutcliffe *et al.*, 1996 b ; Lim 2002). For amplification of *erm A*, *mef A* genes, PCR mixture was composed from 2.5 microliters of template DNA and were

mixed with PCR mixture that composed from 12.5 μ l of GoTaq®Green Master Mix, 2.5 μ l from each primers of *erm A* and *mef A* and 5 of D.D water to get final volume of 25 μ l. The program, for *mef A* the reactions mixtures included an initial denaturation at 93 °C for 3 min. consisted of 35 cycles of 93 °C for one min, specific annealing temperature 52 °C for one min and a final extension at 72 °C for 5 min in a Thermal Cycler , whereas for *erm (A)* the reactions mixtures included an initial denaturation at 95 °C for 5 min, specific annealing temperature 54 °C for one min and a final cycle of primer extension at 72 °C for 5 min. The thermocycler reaction conditions for each primer as fallow pair were calculated on the basis of the annealing temperature and the length of the product size. PCR amplified DNA was analyzed on 0.8 to 1% agarose gels by electrophoresis. Concentration and purity of DNA by Nano drop system. The phylogenetic group to which the *S. epidermidis*, *S. aureus*, *Strp. pyogenes* strains belonged was determined by a PCR-based method.

Table1. Primer sequences and predicted sizes of the amplified products of PCR

Primer		Oligonucleotide sequences (5`-3`)	No. of base pair	Size of amplicons
<i>erm A</i>	F	5- GTT CAA GAA CAA TCA ATA CAG AG – 3	23	421
	R	5` - GGA TCA GGA AAA GGA CAT TTT AC – 3`	23	
<i>mef A</i>	F	5` - AGT ATC ATT AAT CAC TAG TGC – 3`	21	348
	R	5` - TTC TTC TGG TAC TAA AAG TGG – 3`	21	

RESULTS AND DISCUSSION

Activation of Bacterial isolates

Twenty Isolates of *S. aureus*, nine isolates of *S. epidermidis*, one isolate of *S. saprophyticus*, one isolate of *S. albus*, eight isolates of *Strep. pyogenes*, one isolate of *Strep. agalactiae*, isolated from urine, blood, sputum, wound, vagina, pharynx were activated. Diagnosis was confirmed mediated Vitek2 system.

Susceptibility Testing

The results showed the proportion of *S.aureus* resistance to antibiotics of erythromycin, azithromycin and clindomycin was 45%, 45% 25% respectively (Table 2). There are some studies have highlighted the high resistance to azithromycin where that Aktas *et al.*, (2011) in Turkey found that *S. aureus*

resistant to azithromycin with ratio amounted to 96%, while erythromycin 96.1% and for clindomycin 55.1%, which was also higher than the proportion of resistance to the compounds of Macrolide in the current study. 25.5 % of *S.aureus* was resistant to clindomycin, these results agreed with Al-Hasani (2011) pointed out ratio of resistance of clindomycin was 31.2%. As for *Staphylococcus* CO-agulase negative (CONS), *S. epidermidis* which constitute the bulk of them in the current study, the percentage of resistance to erythromycin was 66.6%, while for azithromycin was 44% and for clindomycin was 22.2%, the proportion of resistance of azithromycin was less than results of Hafeth (2010) demonstrated ratio was 87.2%. As for the results of clindomycin did not agree with Al-Hasani (2011) who found all isolates were sensitive to this antibiotic with 100%. The resistance of erythromycin and azithromycin by *Strep. pyogenes*, which constitute the bulk of the genus of *Streptococcus* reached 25 % and 37.5 % respectively, whereas all isolates sensitive to clindomycin as shown in (Table 2), these results of the current study agreed partly with Younge *et al.* (2004) who found resistance of erythromycin was 19%. As for clindomycin the ratio agreed with Moriangenthem and Gurung (2013) found all isolates of *Strep. pyogenes* were sensitive to this antibiotic with 100%, in general that resistance of clindomycin is less than the resistance ratios in most countries of the world. Ciftci *et al.* (2003) found as no more than 3% in Turkey, and Grivea *et al.* (2012) point out that the resistance of clindomycin in Spain 0%. Results show that all bacterial isolates possess resistance to most antibiotics used in this study and varying rates, the results showed that the percentage of erythromycin, azithromycin and clindomycin by *Staphylococcus* spp were 51.6%, 45.1% and 22.5% respectively. As for *Streptococcus* spp. the proportion of resistance to erythromycin, azithromycin and clindomycin were 25.5%, 25.5% and 0.0% respectively. The reason of resistance of *Staphylococcus* spp. and *Streptococcus* spp. for erythromycin, azithromycin and clindomycin to the presence of genes located on plasmids or transposal elements which responsible for this resistance, there are two main ways to resist macrolide group during modulating ribosomal target site by producing 23 rRNA methylase encoding mediated by a gene *erm* including *erm* (A) and *erm* (B) and *erm* (C), which the resulting compounds macrolide resistance and lincosamides and Streptogramin B (MLSB Phenotype) or through acquisition *mef* (A) gene as the gene coding for the construction of efflux pump protein by the expulsion of the macrolide antibiotics outside bacterial cell (Zmantar *et al.*, 2011).

Table 2. Percentage of the sensitivity of antibiotics by *Staphylococcus* spp. and *Streptococcus* spp

Antibiotics Isolates	Erythromycin			Azithromycin			Clindomycin		
	S %	I %	R %	S %	I %	R %	S %	I %	R %
<i>Staphylococcus aureus</i>	50 (10)	5 (1)	45% (9)	45 (9)	10 (2)	45 (9)	70 (14)	5 (1)	25 (5)
<i>Staphylococcus epidermidis</i>	33.3 (3)	0	66.6% (6)	55.5 (5)	0	44.4 (4)	55.5 (5)	22.2 (2)	22.2 (2)
<i>Streptococcus pyogenes</i>	75 (5)	12.5 (1)	25% (2)	62.5 (6)	0	37.5 (2)	62.5 (6)	28.5 (2)	0
<i>Streptococcus agalactiae</i>	100 (1)	0	0%	100 (1)	0	0	100	0	0
<i>Staphylococcus albus</i>	100 (1)	0	0%	100 (1)	0	0	100 (1)	0	0
<i>Staphylococcus saprophyticus</i>	100 (1)	0	0%	100 (1)	0	0	100 (1)		0

S: Sensitive - I: Immediate - R: Resist - NO. Above: Percentage -NO. Below: NO. of isolates

Detection of isolates resistant to erythromycin

Table 3 shows the existence of a high proportion of isolates resistance to erythromycin, as the 14(46.6%) isolates belonging to the genus *Staphylococcus* spp. can grow on erythromycin with concentration reach 100 ug ml⁻¹, while erythromycin sensitive *Staphylococcus* spp. was 17 isolates with ratio 54.8%. Results indicated presence one isolate (11.11%) of total 9 isolates belonging to the genus *Strep. pyogenes* resistant to erythromycin. Despite the decline in the ratio, however, isolates of *Staphylococcus* spp., which are expected to form the strains MRSA large part may be the cause for many of the infections that require to treatment it processing specific varieties of antibiotics, as these resistant strains of several varieties of antibiotics including macrolide group (Duran *et al.*, 2012).

Table 3. Numbers and percentages of isolates resistant to erythromycin

Isolates	Sensitivity to erythromycin	NO. Of isolates (%)
<i>Staphylococcus</i> spp	Resistant	14(46.66)
	Sensitive	16(53.33)
<i>Streptococcus</i> spp	Resistant	1(11.11)
	Sensitive	8(88.88)

Minimal inhibitory concentration

Depending on the results of Antimicrobial susceptibility test and growth at erythromycin medium, 17 isolates belonging to both *Staphylococcus* spp. and *Streptococcus* spp. was elected, isolated from different clinical sources, these

isolates resistant to all antibiotics under study, so as to determine ability of these isolates to resistance of Macrolide antibiotics through the work of a series of concentrations different to determine the minimum inhibitory concentrations based on CLSI (2012). Results in Table 4 indicate that the values of minimum inhibitory concentrations of erythromycin ranged between 16-64 $\mu\text{g ml}^{-1}$ for isolates of *Staphylococcus* spp., these results converged with the results of Richter *et al.*, 2011 whom found MIC of erythromycin is $\geq 64 \mu\text{g ml}^{-1}$, and those results also partially converged with results Aktas *et al.*, (2007) whom pointed out that values of MIC for erythromycin $\geq 128 \mu\text{g ml}^{-1}$.

Table 4. Minimum inhibitory concentrations of erythromycin

No. of Isolates	Isolates	MIC Erythromycin	No. of Isolates	Isolates	MIC Erythromycin	No. of Isolates	Isolates	MIC Erythromycin
Break point ≥ 64								
3	<i>Streptococcus aureus</i>	≥ 64	11	<i>Streptococcus aureus</i>	≥ 32	22	<i>Streptococcus pyogenes</i>	≥ 64
6	<i>Staphylococcus aureus</i>	≥ 64	12	<i>Streptococcus epidermidis</i>	≥ 64	25	<i>Streptococcus epidermidis</i>	≥ 64
7	<i>Staphylococcus aureus</i>	≥ 64	13	<i>Staphylococcus aureus</i>	≥ 32	26	<i>Streptococcus epidermidis</i>	≥ 32
8	<i>Staphylococcus aureus</i>	≥ 64	14	<i>Staphylococcus aureus</i>	≥ 64	27	<i>Streptococcus epidermidis</i>	≥ 64
9	<i>Staphylococcus aureus</i>	≥ 64	16	<i>Streptococcus epidermidis</i>	≥ 64	31	<i>Streptococcus pyogenes</i>	≥ 64
10	<i>Staphylococcus aureus</i>	≥ 64	21	<i>Streptococcus epidermidis</i>	≥ 64			

Staphylokinase and Streptokinase Production

The results (Table 5) indicate to 13(65%) isolates of *S. aureus* produced Staphylokinase, 6(66.6%) isolates of *S. epidermidis* able to produce the same enzyme. *S. albus* and *S. saprophyticus* produced Staphylokinase with ratio 100% Those results agreed with the results of the study conducted by Devi *et al.*, (2012) whom found 7 out of 12(58%) isolates belong to the *Staphylococcus* spp produced this enzyme. While the results showed that 3 (37.5%) isolates able to produce Streptokinase enzyme, while *Strp. agalactiae* could not produce streptokinase (Table 5). The ratio was lower than of the results obtained by Razak and Al-Jebori (2012) found that 66.6% of its isolates able to produce this enzyme.

Table 5. Percentage of *Staphylococcus spp* and *Streptococcus spp.* producing Staphylokinase and Streptokinase

Staphylokinase production		
Isolates	No. of Isolates	No. of isolates producing enzyme (%)
<i>Staphylococcus aureus</i>	20	13(65)
<i>S.epidermidis</i>	9	6 (66.6)
<i>Staphylococcus albus</i>	1	1(100)
<i>Staphylococcus saprophyticus</i>	1	1(100)
Streptokinase Production		
<i>Streptococcus pyogenes</i>	8	3(37.5)
<i>Streptococcus agalactiae</i>	1	0(0)

Detection of genes *erm A* and *mef A* using PCR technique

The results showed that the purity ranged from 1.91 to 2, and the concentrations ranged between 5.993 ng μl^{-1} and 93.5 ng μl^{-1} . The products of PCR technique were rolled on gel electrophoresis with concentration of 1%, as observed one band on same level, after exposing the gel to ultraviolet light, these results induct the primer linkage with its sequence complementary, and notice do not appear any band for the second track which negative control sample comparing bands with DNA Ladder.

The results showed the size of band similar to expected sizes, were a (412 bp) *erm (A)*, (348 bp) for *mef(A)* gene and also agreed with result of Sutcliffe *et al.* (1996 a) and Lim (2002). The results of the current study showed the presence of a high percentage of isolates possess both or one of the genes, with 5 isolates have *erm (A)* gene 33.33% return of *Staphylococcus spp*, results inducted a high proportion of isolates of *Staphylococcus spp* possess the *erm A* gene, 5 out of 15 (33.33%), divided by 4 isolates 80% belong to *S. aureus*, one (20%) isolate belonging *S. epidermidis* Table 6 and Figure 1. The results also showed that 7(46.6 %) isolates belonging to *Staphylococcus spp* possess the *mef A* gene, divided by 5 isolates 71.4 % belong to *S. aureus*, 2 (28.5 %) isolate belonging to *S. epidermidis*. One out of two of *Strep. pyogenes* has been detected, results showed that one isolate possesses *erm A* gene, while another has *mef A* gene (Table 6) and (Figure 2, 3).

Table 6. Numbers and percentages of isolates under study containing the genes *erm A* and *mef A* using the technique (PCR)

Genes	The number of isolates containing genes		
	<i>Staphylococcus</i> spp		<i>Streptococcus</i> spp
<i>erm A</i>	5(33.33%)		1 (50%)
	<i>S. aureus</i> 80%	<i>S. epidermidis</i> 20%	<i>Strep. Pyogenes</i> 50%
<i>mef A</i>	7(466.%)		1(50%)
	<i>S.aureus</i> 71.4%	<i>S.epidermidis</i> 28.5%	<i>Strep. Pyogenes</i> 50%

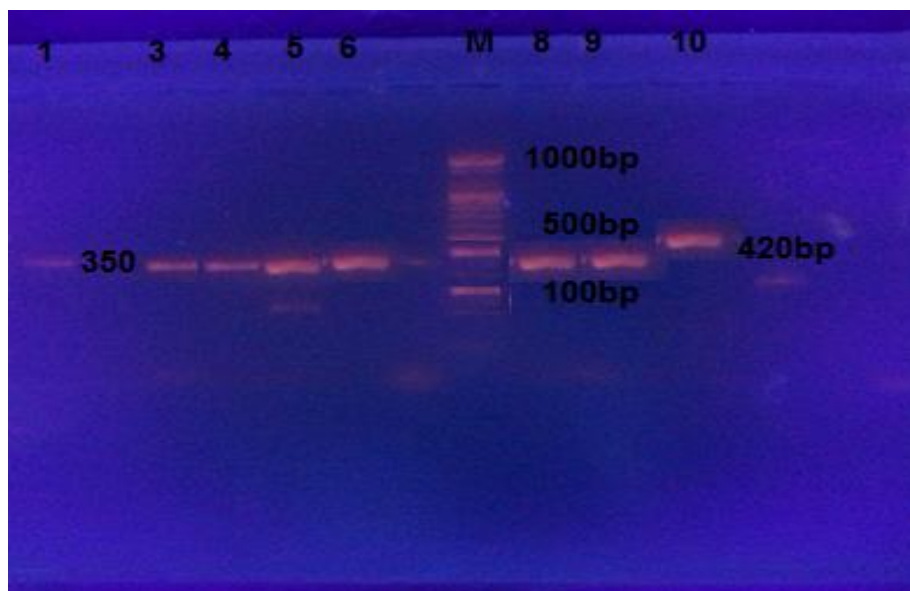


Figure 1. Gel electrophoresis and Ethidium bromide staining to detect genes *erm (A)* and *mef (A)* by using PCR technique, concentration of agarose (1%) ,of 80 volts 45 minutes. Lanes 1, *mef (A)* of *S. epidermidis* ; 2,*mef(A)*of *S.aureus*;3, *mef(A)* of *S.aureus*;4, *mef(A)* of *S.pyogenes*;5, *mef(A)* of *S.aureus*; *mef (A)* of *S.aureus* 6, ; M, Leader DNA (1000 bp) 8, *mef(A)* of *S.epidermidis* ;9, *mef(A)* of *S.epidermidis*;10,*erm (A)* of *S.aureus*. The PCR was carried out in duplicate.

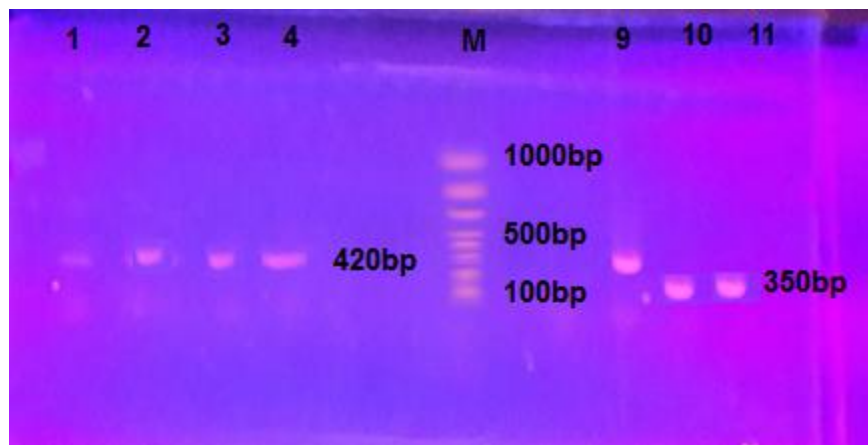


Figure 2. Gel electrophoresis and Ethidium bromide staining to detect genes *erm* (A) and *mef* (A) by using PCR technique, concentration of agarose (1%), of 80 volts 45 minutes. Lanes 1, *erm* (A) of *Strep. pyogenes* ; 2, *erm* (A) of *S.aureus* ;3, *erm* (A) of *S.aureus*; 4, *erm* (A) *S.aureus*; M, Leader DNA (1000 bp 9, *erm*(A) of *S.epidermidis*; 10, *mef* (A) of *S.aureus*; *mef* (A) of *S.aureus*;11 *mef* (A)*S.epidermidis*. The PCR was carried out in duplicate.

It was noted that some isolates in the current study did not show any band through using gel electrophoresis technique, although that it resistant to erythromycin, it may come back to possess these isolates for resistance genes located on a small plasmid, sometimes may be lose this plasmid (Zmantar *et al.*, 2011).

The results partially converged with Aktas *et al.*, (2011) in Turkey found that 50% of isolates of *S. aureus* containing the gene *erm A*, while in regards to *S. epidermidis* far less than reached his results of the current study where the ratio reached *erm* (A) 8.9%. Aktas (2011) pointed out that there are disparities in the distribution of resistance genes in the different countries of the world or the disparity of the hospital may be because randomly use of erythromycin in each country as well as to the person's age and the sample source.

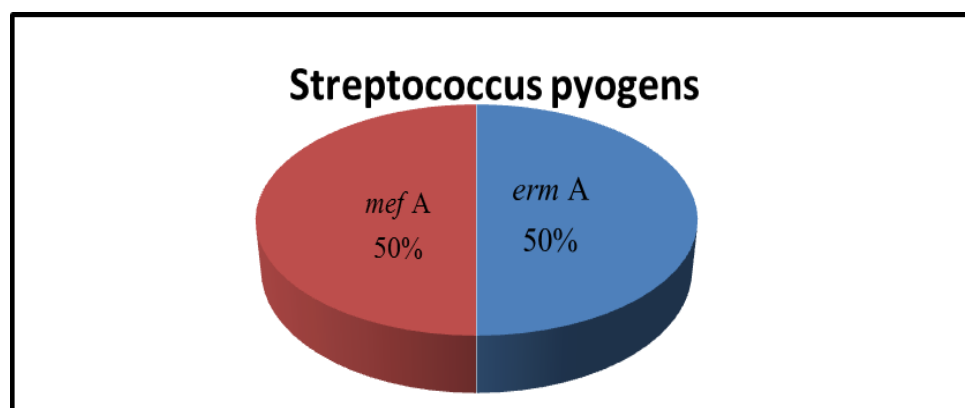


Figure 3. The percentages for the presence of genes in *Streptococcus pyogenes*

The results agree with Duran *et al.*, (2012) in a study by the Mustafa Kemal University in Turkey who pointed out that 33% of the total isolates of *S.aureus* possess the gene *erm A*, As for *S.epidermidis* results converged partly with the results with same researcher who found 11.4% of its Isolates possess the gene *erm A*, also converge with the results of Chaieb *et al.*, (2007) in Tunisia since concluded that 12.5% of the isolates of *S. epidermidis* possess the gene *erm A* but differ with him in gene *mef A* as to reach all of its Isolates do not have this gene and 0%. As for *Strep. pyogenes* products have shown to detect the presence of a single gene 50% , these results Partially agree with López *et al.*, (2012) who conducted studies (1994- 2006) in Spain since reached 35% of the *Strep. pyogenes* isolates possess the gene *erm (A)* while the results of the current study did not agree with López *et al.*, (2012) for the *mef (A)* gene which found that 89% of its Isolates possess this gene. PCR technique was able to give a high-resolution results and the sensitivity of quality as one of the main diagnostic techniques.

CONCLUSION

This study evaluated that *Staphylococcus* spp and *Streptococcus* spp having resistance for Macrolide group and there is relationship between *erm (A)* and *mef (A)* genes and resistance of macrolide.

REFERENCES

- Al-Hasani, H. M. H. 2011. Comparative Study between Methicillin-Resistant Coagulase Positive and Negative Staphylococci. M. Sc. thesis, College of Science, University of Baghdad
- Aktas, A., A. Aridogan, C. B. Kayacan, and B. Aydin. 2011. Resistance to Macrolide, Lincosamide and Streptogramin antibiotics in *Staphylococci* isolated in Istanbul, Turkey. *J. Microbiology*. 45(4): 286-290.
- Amezaga, M. R., P. E. Carter, P. Cash, and H. McKenzie. 2002. Molecular epidemiology of erythromycin resistance in *Streptococcus pneumoniae* isolates from blood and noninvasive sites. *J. Clin. Microbiol.* 40: 3313–3318.
- Chaieb, K, T. Zmantar, O. Chehab, O. Bouchami, A. Ben Hasen, K. Mahdouani and A. Bakhrouf. 2007. Antibiotic resistance genes detected by multiplex PCR assays in *Staphylococcus epidermidis* strains isolated from dialysis fluid an needles in a dialysis service. *Jpn. J. Infect. Dis.* 60: 183-187.
- Ciftci, E, D. Ulker, G. Haluk, D. A. Ahmet, and I. Erdal. 2003. Antibiotic susceptibility of streptococcus Pyogenes strains isolated from throat

- cultures of children with Tonsillopharyngitis. *Journal of Ankara Medical School*. 25(1): 15-20.
- Clinical and Laboratory Standards Institute (CLSI). 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. 32(8). CLSI, Wayne, Pennsylvania, USA.
- Cosgrove, S. E., G. Sakoulas, E. N. Perencevich, M. J. Schwaber, A. W. Karchmer and Y. Carmeli. 2003. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis*. 36: 53–59.
- Cruickshank, R., J. P. Duguiol, B.P. Marmion, and R. H. A. Swain. 1975. Medical Microbiology 14th ed. Churchill Living Stone Edniburg, London and New York.
- Devi, C. Subathra, Dipti Sinha, Vaibhav Sharma and V. Mohansrinivasan. 2012. Screening for Staphylokinase producing *Staphylococcus* spp. from different environmental samples. *Asian Journal of Pharmaceutical and Clinical Research*. 15, suppl4.
- Duran, N. B. Ozer, G. G. Duran, Y. Onlen, and C. Demir. 2012. Antibiotic resistance genes and susceptibility patterns in *Staphylococci*. *Ind. J. Med. Res*. 135: 389-396.
- Emori, T. G. and R. P. Gaynes. 1993. An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev*. 6: 428–442.
- Farrell, D. J., K. P. Klugman and M. Pichichero. 2007. Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *Pediatr. Infect. Dis. J*. 26: 123–128.
- Fung-Tomc, J.C., J. Clark, B. Minassian, M. Pucci, Y. Tsai, E. Gradelski, L. Lamb, I. Medina, E. Huczko, B. Kolek, S. Chaniewski, C.I Ferraro, T. Washo and D. P. Bonner. 2002. *In Vitro* and *In Vivo* Activities of a Novel Cephalosporin, BMS-247243, against Methicillin-Resistant and -Susceptible *Staphylococci*. *Antimicrob. Agents Chemother*. 46(4): 971-976.
- Gill, S. R., D. E. Fouts, G. L. Archer, E. F. Mongodin, R.T. DeBoy, J. Ravel, I. T. Paulsen, J. F. Kolonay, L. Brinkac, M. Beanan, R. J. Dodson, S. C. Daugherty, R. Madupu, S. V. Angiuoli, A. S. Durkin, D. H. Haft, J. Vamathevan, H. Khouri, T. Utterback, C. Lee, G. Dimitrov, L. Jiang, H. Qin, J. Weidman, K. Tran, K. Kang, I. R. Hance, K. E. Nelson, and C. M.

- Fraser. 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early Methicillin-Resistant *Staphylococcus aureus* strain and a Biofilm-Producing Methicillin-Resistant *Staphylococcus epidermidis* strain. *J. Bacteriol.* 187(7): 2426-2438.
- Goossens H., M. Ferech, R., Vander Stichele, M. Elseviers. 2005. Outpatient antibiotic use in Europe and association with resistance: a crossnational database study. *Lancet.* 365: 579–87.
- Grivea, I. N., A. Sourla, E. Ntokou, Chryssanthopoulou, G. Alexandra, D. C. Tsantouli and G. A. Syrogiannopoulos. 2012. Macrolide resistance determinants among *Streptococcus pneumoniae* isolates from carriers in Central Greece. *BMC Infectious Diseases.* 12: 255.
- Hafeth, A. A. 2010. In vitro antibiotic susceptibility and biofilm formation to *Staphylococcus epidermidis* isolates from health care workers in Al-Hussein teaching hospital in Al-Nasserya city. *J. of the Coll. of Educa. For Pur. Sci.* 2(1): 5-9.
- Hoban, D. J., A. K. Wierzbowski, K. Nichol, and G. G. Zhanel. 2001. Macrolide-resistant *Streptococcus pneumoniae* in Canada during 1998–1999: prevalence of *mef(A)* and *erm(B)* and susceptibilities to ketolides. *Antimicrob. Agents Chemother.* 45:2147–2150.
- Kamarulazaman, N. Parasakthi, P. H. Van, T. So, T. K. Ng and the ANSORP Study Group. 2004. Macrolide resistance and genotypic characterization of *Streptococcus pneumoniae* in Asian countries: a study of the Asian Network for Surveillance of Resistant Pathogens (ANSORP). *J. Antimicrob. Chemother.* 53: 457–463.
- Leclercq R. 2002 Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.*34: 482–92. doi:10.1086/324626
- Lim, J. A., A. R. Kwon, S. K. Kim, Y. Chong, K. Lee, and E. C. Choi. 2002. Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. *J. Antimicrob. Chemother.* 49: 489–495.
- López, V. R., S. D. Valdezate, P. Álvarez, Villalón, M. J. Medina, C. Salcedo, and J. A. Sáez-Nieto. 2012. Molecular epidemiology, antimicrobial susceptibilities and resistance mechanisms of *Streptococcus pyogenes* isolates resistant to erythromycin and tetracycline in Spain (1994–2006). *BMC. Microbiol.* 12(215): 1-11.

- Malhotra-Kumar S., C. Lammens, S. Coenen, K. Van Herck, H. Goossens. 2007. Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. *Lancet*. 369: 482–90.
- Malhotra-Kumar, S., A. Mazzariol, L. Van Heirstraeten, C. Lammens, P. de Rijk, G. Cornaglia. 2009. Unusual resistance patterns in macrolide-resistant *Streptococcus pyogenes* harbouring *erm(A)*. *J. Antimicrob Chemother*. 63: 42–6.
- Moirangthem, A. and K. Gurung. 2013. Bacteriological analysis and its Antibiogram profile of pharyngitis cases from the patients attending hospital. Sikkim. India. *BMJ. J.* 2(1): 10-13.
- Monaco, M., R. Camilli, F. D'Ambrosio, M. Del Grosso, and A. Pantosti. 2005. Evolution of erythromycin resistance in *Streptococcus pneumoniae* in Italy. *J. Antimicrob. Chemother*. 55: 256–259.
- Ragamohan, G. and Kanak L. Dikshit. 2000. Role of the N-terminal region of Staphylokinase (SAK): Evidence for the participation of the n-terminal region of (SAK) in the enzyme substrate complex formation. *FEBS Letters*. 474: 151-158
- Razak, M. S. and R. F. Al-Jebori. 2012. Molecular study of staphylokinase enzyme and characterization of some virulence factors in *Streptococcus pyogenes*. *Med. J. Baby*. 9(1): 74-83.
- Reinert, R. R., A. Ringelstein, M. van der Linden, M. Y. Cil, A. Al-Lahham and F. J. Schmitz. 2005. Molecular epidemiology of macrolide-resistant *Streptococcus pneumoniae* isolates in Europe. *J. Clin. Microbiol*. 43: 1294–1300.
- Sutcliffe, J., T. Grebe, A.T Kamradt, and L. T. Wondrack. 1996a. Detection of Erythromycin-Resistant Determinants by PCR. *Antimicro. Ag. and Chemoth*. 40(11): 2562–2566.
- Sutcliffe, J., A. T. Kamradt, and L. Wondrack. 1996b. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. *Antimicro. Ag. and Chemoth*. 40(8): 1824-1817.
- Tankhiwale, S. S., S. V. Jalgaonkar, S. Ahamad, U. Hassani. 2004. Evaluation of extended spectrum beta lactamase in urinary isolates. *Indian J. Med. Res*. 120:553–556.
- Tiemersma, E. W., S. L. Bronzwaer, O. Lyytikainen, J. E. Degener, P. Schrijnemakers, N. Bruinsma, J. Monen, W. Witte and H. Grundman.

2004. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. European Antimicrobial Resistance Surveillance System. *Emerg. Infect. Dis.* 10: 1627–1634.
- Young, U. H, I. H. Jang, G. Y. Hwany, M. K. Lee, K. J. Yoon and H. Y. Kim. 2004. Antimicrobial susceptibility and macrolide resistance genes of β -hemolytic streptococci in korea. *Antimicro. Ag and Chemother.* 48(7): 16 – 27 .
- Zmantar, T. B. Kouidhi, H. Miladi and A. Bakhrouf. 2011. Detection of macrolide and disinfectant resistance genes in clinical *Staphylococcus aureus* and coagulase-negative *Staphylococci*. *BMC. Resea.* 4(453): 2 – 9.

الكشف الجزيئي للجين *erm(A)* و *mef(A)* في *Staphylococcus spp.* و *Streptococcus spp.* المقاومة لمضادات المايكروبيد والمعزولة من مصادر سريرية مختلفة

هادي رحمن رشيد الطائي

قسم علوم الحياة - كلية العلوم - جامعة ديالى - ديالى، العراق (drhadialtaai@yahoo.com)

المستخلص

نشطت العزلات البكتيرية *Staphylococcus epidermidis*, *Staphylococcus aureus* ، *Streptococcus pyogenes* ، *Staphylococcus saprophyticus* ، *Staphylococcus albus* و *Streptococcus agalactiae* والمعزولة من مصادر سريرية مختلفة. اظهرت نتائج فحص الحساسية ان *Staphylococcus aureus* قد قاومت مضادات erythromycin و azithromycin و Clindomycin بنسب 45% و 45% و 25% على التوالي، بينما كانت مقاومة *Staphylococcus epidermidis* لنفس المضادات بنسب 66.6% و 44% و 22.2% على التوالي. مقاومة المضادين erythromycin ، azithromycin من قبل *Streptococcus pyogenes* وصلت الى 25% ، 37.5% بينما جميع العزلات حساسة لـ Clindomycin ونسبة 100%. اوضحت النتائج ان التركيز المثبط الادنى MIC لـ erythromycin كان ≥ 64 - 32 مكغم مل⁻¹. ان 13 (65%) من عزلات *Staphylococcus aureus* منتجة لانزيم Staphylokinase وان 6 (66%) *Staphylococcus epidermidis* منتجة لنفس الانزيم. بينت النتائج ان 3 (37%) *Streptococcus pyogenes* انتجت انزيم Streptokinase ولكن *Streptococcus agalactiae* لم تستطع انتاج الانزيم. نتائج تقنية PCR كشفت تواجد الجين *erm(A)* في 33.3% من عزلات *Staphylococcus spp* و 50% من عزلات *Streptococcus spp.* بينما بينت النتائج تواجد جين *mef(A)* في 46.6% من عزلات *Staphylococcus spp* و 50% من عزلات *Streptococcus spp*. بينت النتائج هناك تنوع في النمط المظهري مقارنة بالنمط الوراثي.

الكلمات المفتاحية: المكورات العنقودية، المسبقيات الكروية، مضادات المايكروبيد، جين *erm(A)*، *mef(A)*، جهاز بلمرة الدنا.