Biochem. Cell. Arch. Vol. 21, No. 1, pp. 1443-1448, 2021	www.connectjournals.com/bca	*ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2021.21.1443		*eISSN 0976-1772*

MOLECULAR DETECTION OF *ermA*, *ermB* AND *ermC* GENES AMONG METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENTS WITH OCULAR INFECTIONS

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(Received 6 November 2020, Revised 24 January 2021, Accepted 31 January 2021)

ABSTRACT : One hundred and twenty eye swabs were collected from different patients suffering from ocular infections of both sexes admitted to Ophthalmic Unit of Hilla Teaching Hospital from May to October 2020 in Hilla province, Babylon Governorate, Iraq. The results revealed that *Staphylococcus aureus* isolates were detected in (76 out of 120) eye swabs by using standard microbiological and biochemical tests. Antibacterial susceptibility tests have been carried out by using Kirby-Bauer disk diffusion method. The results revealed that (84.2%) were resistant to Cefoxitin, (40.8%) were resistant to Ciprofloxacin, (38.2%) were resistance to Erythromycin, Gentamicin, and Tetracycline, whereas (22.3%) were resistant to Clindamycin. The multiplex PCR technique was achieved to identify the presence of (*mecA*) gene, and the Erythromycin genes such as "*ermA, ermB* and *ermC*" in MRSA isolates by using specific primers. The PCR results showed that among these isolates it was found that (64 out of 76) *Staph. aureus* isolates were MRSA (including *mecA* gene) (84.2%) and 12 isolates were did not contain *mecA* gene (15.8%) is MSSA. On the other hand, about MRSA (64) isolates were shown the prevalence of erythromycin genes. The results showed that the predominance of the *ermC* gene 23.4% (n=15), followed by the *ermA* gene 17.2% (n=11), while *ermB* gene with a slight prevalence 4.7% (n=3). Generally, multiplex PCR assay is speed, very sensitive, and a more suitable method which takes less time than the use of antibiotic susceptibility test for determination of methicillin and ERM resistance genes among *Staph. aureus* isolates.

Key words : *Staphylococcus aureus*, ocular infection, molecular detection, *mecA* gene, *erm* genes.

How to cite : Jundi Alak Mahdi Al-Buhilal, Mohammed Saad and Niran Kadhim F. AL-Rubaey (2021) Molecular detection of *ermA*, *ermB* and *ermC* genes among methicillin resistant *Staphylococcus aureus* isolated from patients with ocular infections. *Biochem. Cell. Arch.* **21**, 1443-1448. DocID: https://connectjournals.com/03896.2021.21.1443

INTRODUCTION

A broad variety of diseases, including bacteremia and pleuropulmonary, skin and soft tissue infections, are caused by *Staphylococcus aureus*. Many forms of ocular infections, including keratitis, conjunctivitis, endophthalmitis and blepharitis, are also responsible for this bacterium. There are several studies about ocular infection caused by (MRSA) isolates. In the past few decades, the infection with MRSA strains have arisen in hospital settings and communities (Chuang *et al*, 2012). Several studies have demonstrated the *Staph. aureus* is the main cause of ocular infections in various countries of the world (Okesola and Salako, 2010). The antibiotic resistance of *Staph. aureus* nowadays is actually a significant problem in the community (Prabhu *et al*, 2011). For the prevention of ocular diseases, antibiotics such as erythromycin and tetracycline have been used for the treatment of *Staph. aureus* (Shanmuganathan *et al*, 2005). *Staphylococcus aureus* isolates are distinguished by resistance to erythromycin, a resistance to other macrolides is also related, and three genes have been identified, including, *ermA*, *ermB* and *ermC* were responsible for this resistance. These genes encoding methylase enzymes in which they play an important role in the modulation of the ribosomal target site contributing to the Lincosamide, Macrolide and Streptogramin type B (MLSB) phenotype (Zmantar *et al*, 2011). The 23S rRNA methylation conferred by *erm* genes prevents the antibiotic from binding to its ribosomal target (Hess and Gallert, 2014). Methylases in the 50S ribosomal subunit decrease the binding of MLSB antibiotics to the target site. Two important genes responsible for MLSB resistance in

Staph. aureus are *ermA* and *ermC*. In methicillin resistant isolates *Staph. aureus*, *ermA* genes are frequently distributed and are produced by Tn554 transposons, while *ermC* genes are mainly responsible for erythromycin resistance among methicillin susceptible isolates and are carried by plasmids (Vandendriessche *et al*, 2011).

The current study aimed to isolate and identify of *Staph. aureus*, the isolates were studied for resistance to some antibiotics and determination the prevalence of methicillin resistant *Staph. aureus* (MRSA) isolates, in addition to detect the Erythromycin resistance genes, including "*ermA*, *ermB*, *ermC*" genes by multiplex PCR technique among MRSA isolates obtained from eye swabs of patients with ocular infection in Hilla province, Babylon Governorate, Iraq.

MATERIALS AND METHODS

Collection and identification of bacterial isolates

In a cross sectional study, during a period from May to October 2020 in Hilla province, Babylon Governorate, Iraq. Eye swabs were collected from 120 different patients suffering from ocular infections of both sexes admitted to Ophthalmic Unit of Hilla Teaching Hospital. These eye swabs from the vitreous, corneas and conjunctiva were examined and the relevant patient information such as age, gender and clinical symptoms were collected. The identification of microorganisms was performed according to standard microbiological methods. All of the culture media used in this study were supplied by HiMedia, Co, India (Sadeghi and Mansouri, 2014). Seventy six isolates were considered as *Staph. aureus* by using standard microbiological tests, such as, Gram's stain, β-hemolysis on blood agar base enriched with 5% sheep blood, catalase reaction, the production of coagulase, DNase and fermentation mannitol salt agar media.

Antibiotic sensitivity test

The Kirby-Bauer disk diffusion method on Mueller Hinton agar was used to determine The susceptibility of isolates to some antibiotics such as: Erythromycin (15 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), and Tetracycline (30 µg) (Melvin *et al*, 2017). Also, All isolates of the *Staph. aureus* were exposed to Cefoxitin (30 µg). According to CLSI guidance, the findings were interpreted. An inhibition zone diameter of ≥ 22 mm was considered as (methicillin sensitive), while ≤ 21 mm was considered as (methicillin resistant).

Genotypic detection of MRSA isolates

MRSA isolates prevalence was achieved by using unique primers and amplicon size to detect the *mecA* gene, which responsible for methicillin resistance as described in Table 1 (Cabrera *et al*, 2010). Template DNA was prepared by using the boiling methods. Generally, in 5 ml of TE buffer, several isolated colonies of overnight growth bacteria were thoroughly suspended and boiled in a water bath for 5 minutes, then isolation the supernatant and used as a DNA template. By adding (12.5µl) of GoTaq ® Green Master Mix (2X) Promega, 5µl DNA prototype, 1.5µl from both forward and reverse primer with final concentration of 1 poml / µl, the final volume was completed to (25µl) by adding nuclease free water to the PCR mixture. PCR condition usually started the process with initial denaturation step were carried out at 95°C for 3 minutes, 35 cycles consisting of a denaturation step at 94°C for 30 seconds, primer annealing at 53°C for 30 seconds. Then extension at 72°C for 30 seconds. Final extension at 72°C for 10 minutes. The PCR product was analyzed on 1% agarose gels, stained with ethidium bromide. Finally, the bands were visualized on UV transilluminator.

Genotypic detection of *ermA*, *ermB* and *ermC* genes in MRSA isolates

The PCR technique was used to determine the prevalence of (*erm A*, *erm B* and *erm C*) genes. The method was done by using specific primers with the amplicon size and the primer sequence was listed in Table 1 as described previously (Martineau *et al*, 2000) with

Table 1 : The oligonucleotide primer sequence.

Genes	The sequence from 5'to 3'	Product size (bp)	Reference
mecA	F-GTAGAAATGACTGAACGTCCGATAA	314	Cabrera *et al* (2010)
	R- CCAATTCCACATTGTTTCGGTCTAA		
ermA	F-TATCTTATCGTTGAGAAGGGATT	139	Martineau *et al* (2000)
	R-CTACACTTGGCTTAGGATGAAA		
ermB	F-CTATCTGATTGTTGAAGAAGGATT	142	
	R-GTTTACTCTTGGTTTAGGATGAAA		
ermC	F-CTTGTTGATCACGATAATTTCC	190	
	R-ATCTTTTAGCAAACCCGTATTC		

some modifications.

Multiplex PCR assays were performed in the final volume completed to 25 µL with sterilized D.W. The PCR mixture contains 5 µL of (5x) buffer reaction, 25 pmol/L each forward and reverse primers of *ermA*, *ermB* and *ermC* genes, 1 U of GoTaq DNA polymerase (Promega, France), 100 µmol/L of each dNTP and (50 ng) of DNA template. The thermal cycling condition usually carried out at 96°C for 3 minutes as initial denaturation, 40 cycles consisting of a denaturation at 94°C for 30 seconds, primer annealing at 56°C for 30 seconds. Then extension at 72°C for 30 seconds. Final extension at 72ºC for 10 minute. The PCR product was analyzed on 1% agarose gels, stained with ethidium bromide. Finally, the bands were visualized on UV transilluminator.

RESULTS

All (120) eye swabs obtained from patients suffering from ocular infections were analyzed to detect the presence of *Staphylococcus aureus* isolates by using standard microbiological tests and biochemical tests. 76 isolates were approved as *Staph. aureus*. Among these isolates it was found that 64 (84.2%) *Staph. aureus* isolates were resistance to Cefoxitin and considered as MRSA, (40.8%) was resistance to Ciprofloxacin, (38.2%) were resistance to Erythromycin, Gentamicin, and Tetracycline, whereas 17 (22.3%) was resistance to Clindamycin (Fig. 1).

Among these isolates 64 (84.2%) of them were MRSA (including *mecA* gene by PCR) and 12 (15.8%) isolates were did not contain *mec*A is MSSA as shown in Table 2 and Fig. 2.

DNA was extracted from all these (64) MRSA isolates and used as template to detect Erythromycin genes (*ermA, ermB* and *ermC*) by using specific primers. The results revealed that the *ermC* gene was the utmost frequent 15 (23.4%), followed by *ermA gene* 11 (17.2%), and *ermB* gene 3 (4.7%) reported as the least frequent detected gene. The results shown in Table 3 and Fig. 3.

Table 2 : Multiplex polymerase chain reaction assay to detect *mec*A gene in *Staph. aureus* isolates.

Multiplex PCR assay results	No. of *Staph. aureus*	Percentage (%)
Positive for *mecA* gene (MRSA)	64	84.2
Negative for *mecA* gene (MSSA)	12	15.8
Total	76	100

Table 3 : Distribution of eye swabs according to Erythromycin genes in MRSA isolates from patients with ocular infections.

Methylases genes	No. of *Staph. aureus* isolates	Percentage (%)
ermA	11	17.2%
ermB	3	4.7%
ermC	15	23.4%
Total	29	45.3%

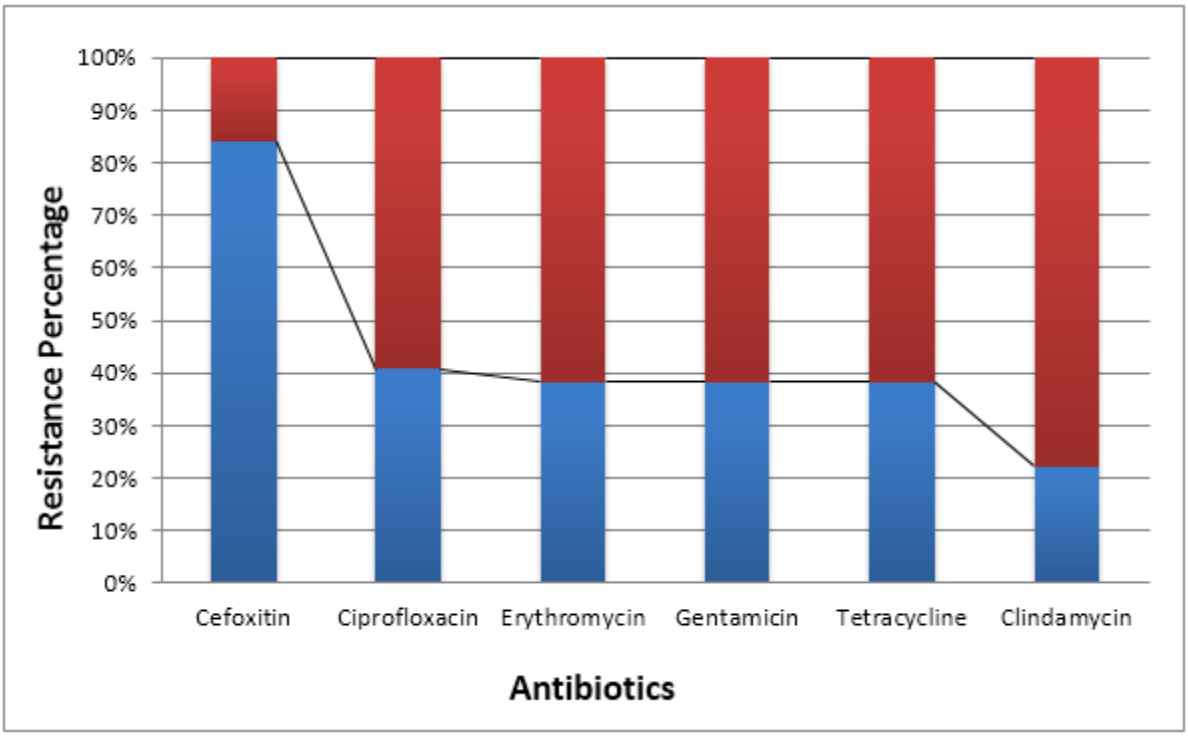

Fig. 1 : Resistance rate of *Staph. aureus* isolates to some antibiotics.

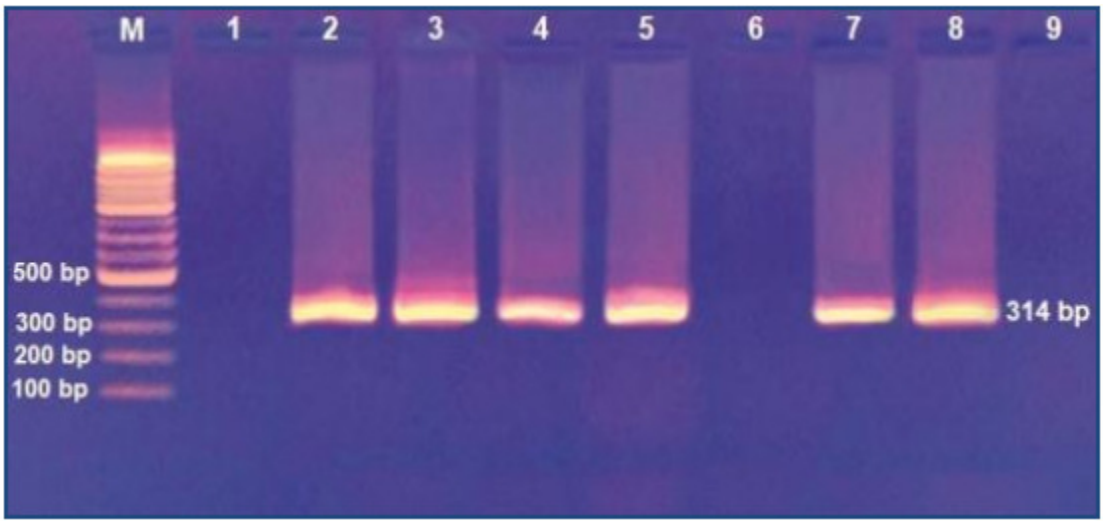

Fig. 2 : Agarose gel electrophoresis of extracting total DNA (*mecA* gene) by using 1% agarose gel at 50 Volt for 1 hour. Lane M: marker with 100 bp ladder. Lane 2-5 and 7-8: positive for *mecA* gene. Lane 1, 6 and 9: negative for *mecA* gene, product size: 314 bp.

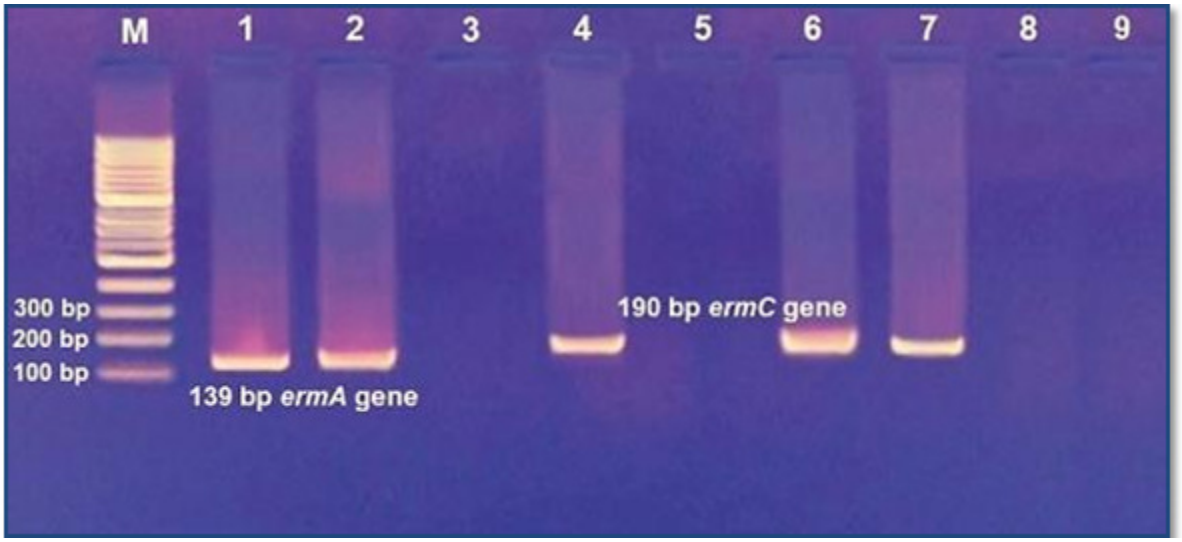

Fig. 3 : Agarose gel electrophoresis of multiplex PCR assay to identify *ermA* and *ermC*) in MRSA strains isolated from eye swabs by using 1% agarose gel at 50 Volt for 1 hour. Lane M: marker with 100 bp ladder. Lanes 1-2: positive for *ermA* gene, product size: 139 bp, while lanes 4 and 6-7: positive for *ermC* gene, product size: 190 bp. Lanes 3, 5, 8, and 9 negative results.

DISCUSSION

Staphylococcus aureus is one of the most important bacteria that has caused numerous problems in treatment. Antibiotic resistance is an important concern in the world and a debatable issue. In recent years, the incidence of resistant staphylococcal infections has increased due to overuse of antibiotics and the transfer of resistance genes (Talebi *et al*, 2019). MRSA is resistant to a wide variety of antimicrobial agents, such as: chloramphenicol, fluoroquinolones, macrolides, tetracyclines, aminoglycosides and lincosamides (Ardic *et al*, 2005).

In the current study, the results showed that *Staphylococcus aureus* isolates were detected in 76 out of 120 eye swabs of patients infected with ocular infections of both sexes, it is the predominant pathogen. This finding is correspondence with previous study in Hilla province, Iraq, reported by Alrubaey *et al* (2008), who found that *Staph. aureus* is the most common bacteria isolated from eye swabs of patients suffering from ocular infection (Blepharitis) of both sexes.

Sixty four (84.2%) *Staph. aureus* isolates were resistant to Cefoxitin and considered as MRSA. The *ermC* gene was detected in an MRSA isolate obtained from patients with external ocular infections. These isolates were also resistant to Ciprofloxacin, Erythromycin, Clindamycin, Gentamicin and Tetracycline. These founding relatively similar with Faridi *et al* (2018) he mentioned that some isolates were resistant to Cefoxitin which is considered MRSA. The *ermC* gene was only detected in an MRSA isolate obtained from patients with external ocular infection. The resistance rate is increasing in *Staph aureus*, that related to their ability of this bacteria

to acquire resistance and overuse usage of antibiotics and the resistance rate varies due to the variation in size of the sample or the year of sampling, Sampling time by the passage of time and the type of antibiotic used. Generally, the antibiotic resistance rate is increasing every year (Talebi *et al*, 2019).

Moreover, the results found that MRSA isolates at a high rate of (84.2%) more than MSSA isolates. These findings must be taken into regard for successful prevention and treatment methods. The previous result study performed in China, that included 519 ocular infections positive to *Staph. aureus*, at a prevalence rate of (52.8%) of MRSA (Hsiao *et al*, 2012). In Europe, However, Morrissey *et al* (2004) found that (22%) of ocular infections caused by MRSA, while the other previous study in Brazil, showed that the low prevalence rate of MRSA was (9.9%) among *Staph. aureus* isolated from ocular infections (Vola *et al*, 2013). These findings showed that the differences in the prevalence of MRSA probably due to the standard microbiological methods, using different kits for the same method or epidemiological developments over time.

Erythromycin Staphylococci resistance is generally associated with resistance to other antibiotic macrolides that are directly mediated by methyltransferases encoded by the three *erm* genes (Zmantar *et al*, 2008). This study has been revealed that *ermC* gene was more prevalent in MRSA isolated from ocular infections followed by the *ermA*, while *ermB* with a slight prevalence. A study carried out by Fasihi *et al* (2017) in Kerman, Iran demonstrated that (*ermA*, *ermB* and *ermC*) genes were associated with the *mecA* gene and MRSA isolates. These findings showed that is probably that *Staph. aureus* isolates from patients suffering from ocular infections more likely to have a link between the *mecA* gene and Erythromycin genes. Also, the findings suggest that plasmids can quickly pass the *ermC* gene to other species, which may be due to local antibiotic policies. Fluit *et al* (2001) found out that on a small plasmid, the *ermC* gene responsible for ERM resistance is located.

CONCLUSION

Our study showed *Staph. aureus* is the predominant bacteria in ocular infections and a growing in the prevalence of MRSA that associated with ocular infections compared to MSSA. Also, MRSA isolates are usually multi-drug resistant, So, the results of antibacterial susceptibility tests should be taken into account when treating eye infections. On the other hand, the detection of *mecA* gene in *Staph. aureus* and ERM resistance genes in MRSA isolates by using of multiplex PCR technique was considered to be speed, very sensitive, and a more suitable method which generally takes less time than the use of antibiotic susceptibility test. Thus, by detecting resistant isolates and applying sufficient care, the spread of resistant isolates can be somewhat stopped.

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