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Detection of *mecA* and *ermC* genes in *S. epidermidis* isolates among acne patients

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***Abstract*— The purpose of the recent study was to profile of two resistance genes (*mecA* and *ermC*) in *S.epidermidis* which isolated from acne patients. In the study, 150 samples of acne from acne sufferers were collected between August and September 2022 in a private clinical place in the Thi-Qar province of Iraq. The *S. epidermidis* was only found in (40%) isolates. Microscopical examination, morphological characterization , diverse biochemical testes, and accurate identification with Viteks-system were used to recognize *S. epidermidis* isolates.. The PCR technique results recorded that (90%) of *S. epidermidis* isolates had *mecA* genes and low percentage of *ermC* (10%).**

Keywords— *S. epidermidis*, *mecA* gene, *ermC* gene

I. Introduction

Acne vulgaris is a Pilosebaceous unit illness, and it is also known as acne vulgaris usually observed in adolescence and it varied in severity from person to person. Acne vulgaris appeared widely in face, chest, and back areas of patients due to the dense sebaceous follicles in these areas. About 80–90% of adolescents in the Western world have acne during adolescence, and this percentage decreases in the rural societies. Acne may occur on inflammatory or non-inflammatory forms. (1,2). The disease had four main causes: infections, hormones and genetics. The *S. epidermidis* is a one of normal flora and it frequently appears on the skin and in mucous membranes. However, *S. epidermidis* has the possibility causes infections in particular condition, since it lives on human skin and mucous membranes in large number (3). The s.epidermidis Lacking the coagulase enzyme and distinguishes them from coagulase staphylococci positive such as *S. aureus* (4). The resistance of bacterial strains to antibiotic makes the CoNS infections (methicillin-resistant *S. epidermidis* (MRSE) difficult to be treated (5). Staphylococcal isolates with methicillin resistance acquired and integrated the staphylococcal cassette chromosome-mec (SCCmec), contains the methicillin resistance gene *(mec* A), that codes to penicillin binding protein2a (PBP2a) (6). *Erm* genes are mostly responsible for erythromycin resistance in different Staphylococcus spp (7). The *ermA and ermC* are the most common genes for resistance to MLSB in staphylococci spp (8).). The *ermC* located on a mobile genetic portion of a plasmid with size of 3.7 kb (9). The goal of this present study was to detect the two resistance genes (*mecA* and *ermC*) in *S.epidermidis* isolated from acne patients.

II. Material and Methods

One hundred and fifty samples from patients 'acne were obtained from Thi-Qar province during August and September of 2022. Transport media used to collect samples from patients' acne. closed comedones and papules were mined by making a scratch in the lesion surface through a lancet, then sketch out the insides with compression on it (10).

A. *S. epidermidis identification*

All samples of acne were incubated on different medium. Manitole salt agar was differential and selective media that was used for isolation and identification the *Staphylococcus* spp. The *S. epidermidis* isolates were inoculated in differential media, Blood agar, Chrom agar, to identify the colonies shape, color, and pigments. Then all the plates were incubated at 37°C for 24 hours. Formerly, *S. epidermidis* colonies were used for performing additional biochemical testes to demonstrat the isolates' identification. Viteks compact system was used to the identify *S. epidermidis* exactly.

B. *Polymerase chain reaction of S. epidermidis isolates*

For extracting the DNA from all isolates of *S.pidermidis*, Presto™ Mini gDNA Bacteria Kit was used. Amplification of the *mec*A and *erm*C genes were done using primers described in Table(1).

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`Table (1): Primer sequences of *ermC* and *mecA* genes

NO	Primer	Primer Sequences (5´- 3´)	Size Product	Refernces
1	*mecA*	F:TCCAGATTACAACTTC ACCAGG	162bp	11
		R:CCACTTCATATCTTGT AACG		
2	*ermC*	F:ATCTTTGAAATCGGCT CAGG	295bp	12

The entire volume of the PCR tubes was 50µl, and it was made up of the following: 10µl of Master Mix, 1µl of both the forward and reverse primers that were particular to each gene, 5µl of bacterial DNA, and the remaining volume was filled with nuclease-free water. The thermocycling protocol for the PCR amplification of both genes was described in table (2).

Table (2): Program of *mecA* and *ermC* genes (11,12)

Step	Temperature, °C	Time	Cycle
Initial denaturation	95	3 min	1
Denaturation	94	60 sec	30
Annealing	55	45 sec	
Extension	72	60 sec	
Final extension	72	5 min	1

III. Results and Discussion

A. *Bacterial isolation and identification*

Only 60 isolates (40%), of the used samples ,whitecolonies on the mannitole salt agar and blue small colonies on chrom agar, were non-mannitole fermenters and recognized as *S. epidermidis*, as shown in Fig. (1). However, only 10/150 (6.7%) of the isolates did no grow on this medium, and (80/150; 53.3%) of them were classified as different bacterial species. Statistically, there were a significant difference amongst *S.epidermidis* and others *Staphylococcus* at (P≤ 0.05).

Fig. (1): The appearance of *S. epidermidis* on A- chrom agar B- manitol salt agar

Totally *S. epidermidis* isolates were identified by biochemical testes as Catalase, Novobiocin sensitivity and Voges-Proskauer tests gave positive results, while Citrate ,Coagulase, DNase, Indol and Methyl red gave negative results. The *S. epidermidis* isolates diagnosed based on Vitek system testes to identify precisely of the desired bacteria.

B. *Molecular diagnosis*

The results of PCR showed 90% of *S. epidermidis* isolates giving positive results for *mecA* gene. Despite the fact that the *ermC* gene finds in slightly percentage just 10% , as represented in Table (3). Statistically, there was a significant differences among gene distribution at (P≤ 0.05).

Table (3): occurrence of *mecA* and *ermC* genes in *S. epidermidis* isolates

Gene	Positive results No=60	p. value
mec A	54 (90%)	0.00003
ermC	6 (10%)	
** Chi-Square = 36.800		

According to PCR assay of *ermC* gene, the bands shows in Fig. (2) determined the size of *ermC*gene .The size was nearly 295bp. Although, Fig. (3) revealing the size of *mecA* gene was closely 162bp.

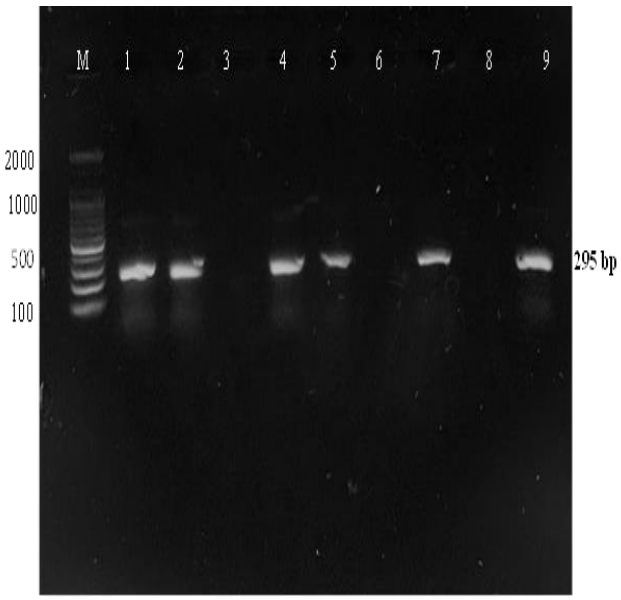

Fig. (2): Agarose gel electrophoresis of *ermC* gene amplification, wherever M: ladder, 1-2,4-5,7,9: positive results; 3:negative control; 6,8 : negative results

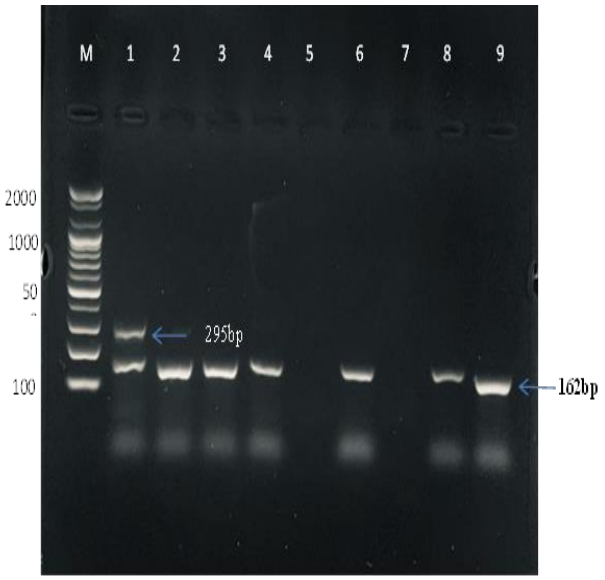

Fig. (3): Agarose gel electrophoresis of *mecA* and ermC genes amplification, where M: ladder, 1: positive results of both genes ,2-4,6,8-9: positive results of mecA gene; 5: negative control; 7 : negative results.

The current data of PCR technique recorded that (90%) of *S. epidermidis* isolates harbored *mecA* gene. The PCR technique was used to identify *Staphylococcus* spp. (13).

In diverse countries, the resistance of *S. epidermidis* to methicillin increased in the world (14). *S. epidermidis* should be resistance to beta-lactam antimicrobial agents. Methicillin-resistant coagulase negative was more resistant to antibiotics than *S. aureus*. The *S. epidermidis* was a tank for mec A and transferred this gene to *S. aureus* through horizontal gene transmission (15).

The present results of *mecA* gene percentage were not integrated with results of (16) displayed the incidence of mecA gene was (61.64%). Also, (17, 18, 19) showed the *S. epidermidis* harbored *mecA* gene in different percentage (64.0%, 70.7%,75.43%), respectively. Different studies indicated a *S. epidermidis* had high percentages of resistance against methicillin and carried *mec*A gene, as (20) showed that the *mec*A gene found in 95.8% of *S. epidermidis* isolates; also the *S. epidermidis* giving positive results of *mec*A gene (93.75%) (21). The studied performed by (22, 23) recorded that (85%,92.2%) of isolates harbored *mecA* gene. Other studies reported a low percentage of *mecA* gene existence, like: (24, 25) indicated (34.4%,10%) of isolates carried the *mecA* gene. The study conducted by (26) confirmed that (61.9%) of *S. epidermidis* isolates had *mecA* gene .

The occurrence of *ermC* gene was 10% in entirely isolates of *S. epidermidis*. Macrolides, lincosamides, and streptogramin B (MLSB) antibiotics, particularly erythromycin and clindamycin were essential medicines for treating methicillin resistant staphylococci infection (27). The *ermC* gene was predominant amongst coagulase negative staphylococci (28). The results of *ermC* gene percentage was near with results of (29, 30, 31) indicated that low percentage of the *ermC* (2.1 %,3.13%, 5.5%), respectively.

Some studies detected high frequency of *ermC* gene in *S. epidermidis* as: (32,33) represented (76%, 66%). While the studies performed by (34, 28) showed 41.3%, 27.3% isolates of the goal bacteria had *ermC* gene, which phenotypically resistance to erythromycin and clindamycin. Therefore, *ermC* gene was the furthermost recurrent gene of erm genes in *S. epidermidis* (43.8%) (35).

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