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 tests, 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 (methylillin-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.epidermidis, Presto™ Mini gDNA Bacteria Kit was used. Amplification of the mecA and ermC genes were done using primers described in Table(1).
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).

### III. RESULTS AND DISCUSSION

#### A. Bacterial isolation and identification

Only 60 isolates (40%), of the used samples, white colonies on the mannitol salt agar and blue small colonies on chrom agar, were non-mannitol fermenters and recognized as *S. epidermidis*, as shown in Fig. (1). However, only 10/150 (6.7%) of the isolates did not grow on this medium, and (80/150; 53.3%) of them were classified as different bacterial species. Statistically, there was a significant difference amongst *S. epidermidis* and others *Staphylococcus* at (P≤ 0.05).

#### 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 (1): Primer sequences of *ermC* and *mecA* genes |
|-------|---------------|-------------------|------------|
| NO   | Primer | Primer Sequences (5´-3´) | Size Product | References |
| 1    | mecA   | F:TCCAGATTACAACTTC ACCAG | 162bp       | 11         |
|      |        | R:CCACTTCATATCTTGT AACG  |             |            |
| 2    | ermC   | F:ATCTTTGAAATCGGCCGGAACG | 295bp       | 12         |

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.

| 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 |

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.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Positive results</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>54 (90%)</td>
<td></td>
</tr>
<tr>
<td>ermC</td>
<td>6 (10%)</td>
<td>0.00003</td>
</tr>
</tbody>
</table>

**Chi-Square = 36.800**

![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](image)

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

Totally *S. epidermidis* isolates were identified by biochemical testes as Catalase, Novobiocin sensitivity and...
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 furthermore recurrent gene of erm genes in S. epidermidis (43.8%) (35).

REFERENCES


