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RESEARCH ARTICLE

MOLECULAR DETECTION OF ANTIBIOTIC RESISTANCE GENE (*MECA*) IN STAPHYLOCOCCUS EPIDERMIDIS LOCAL ISOLATES.

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Abstract

The *mecA* gene is a gene found in bacterial cells. The *mecA* gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other antibiotics. This study is based on the molecular analysis of the antibiotic resistance gene (*mecA*). The polymorphism of the *mecA* gene existing in different *S. epidermidis* have been analyzed in this study. Seventy five isolates (57) of *S. epidermidis* were obtained from eighty four (84) which were collected from three hospitals in Baghdad include Al-Yarmuk General Teaching Hospital, Al-Karama teaching hospital, Ibn-ALbalady teaching hospital. These isolates were collected from clinical sources the samples included, wound swab, burn swab, ear swab and urine. DNA from each sample was isolated and the *mecA* gene was amplified with appropriate primers. The PCR results of 57 *mecA* gene positive *S. epidermidis* gave the band size of amplicons about 500pb. The presence of *mecA* gene seems to be enhanced in biofilm producers and enable *S. epidermidis* strains to show increasing resistance to different groups of antibiotics.

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Introduction:-

S. epidermidis which is known as a natural colonizer of healthy human skin and mucosa, is also a common nosocomial pathogen along with other Coagulase Negative Staphylococci (CONS)[1]. *S. epidermidis* is the most important pathogen, responsible for many indwelling medical device related infections such as; catheter, prosthetic joint, vascular graft, surgical site, central nervous system shunt and cardiac device related ones. Therefore, *S. epidermidis* strains especially emerge as life-threatening pathogens triggering septicemia, meningitis and other serious conditions in medical device using and immunocompromised patient[2, 3, 4, 5]. One of the most important mechanisms making these commensal inhabitants dangerous for medical device using and immunocompromised patients is known as biofilm formation. By means of having ability to adhere to various surfaces and form slimy layer known as biofilm on them[6, 7, 8, 9]. Biofilm is believed to make clinical *S. epidermidis* strains more resistant to administered antibiotics and to host defense mechanisms and highly contributed to cause nosocomial infections (NI) in patients [10].

In clinical practice, *S. epidermidis* has become one of the most significant species among methicillin-resistant coagulase negative staphylococci (CONS). There are various scientific world reports that stated that approximately between 80% and 90% of *S. epidermidis* strains isolated from patients with nosocomial infections (NI) carried the

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mecA gene [11 , 12]. The presence of *mecA* gene seems to be enhanced in biofilm producers and enable *S. epidermidis* strains to show increasing resistance to different groups of antibiotics [13, 14].

The *mecA* gene is a component of a large DNA fragment designated as *mecDNA*. The acquisition of *mecDNA* is considered to be the first genetic requisite for methicillin resistance in staphylococci [15]. The aim of this study was to detect the presence of *mecA* gene in *S. epidermidis* from clinical sources by PCR amplification.

Material and method:-

Sample collection:-

Fifty seven isolates were obtained from 84 samples taken from clinical sources which were collected from three hospitals in Baghdad include Al-Yarmuk General Teaching Hospital , Al-Karama teaching hospital, Ibn-ALbalady teaching hospital. These sample include (wound swab, ear swab, burn swab and urine). All samples were cultured on blood agar and incubated at 37°C for 24 hours. And then on differential media mannitol salt agar *S. epidermidis* is not color change.

All the isolates were identified on the basis of colony morphology, biochemical test catalase and coagulase test. Then identified using a commercial biotyping system (api STAPH, bioMerieux, Inc., Hazelwood, MO) [16, 17, 18]. The strains were stored in brain heart Infusion

(BHI) broth with 20% of glycerol at -20°C. The working cultures of the isolates were prepared in BHI broth at 37°C for 18 h. Chromosomal DNA from the *S. epidermidis* strains isolated from different samples was extracted

Antibiotic Susceptibility Test:-

The Kirby-Bauer's disc diffusion method using methicillin 5 µg disc on Muller-Hinton agar incubated overnight at 37°C was done for the detection of methicillin-resistant strains of *S. epidermidis* according to the zone that form around antibiotic disc.

DNA extraction:-

DNA extraction using Genomic DNA extraction Kit, concentration and purity were determined using Nano drop 1000 spectrophotometer at 260/280nm.

mecA Gene Detection:-

Staphylococcus epidermidis isolates were investigated for the presence of antibiotic resistance gene (*mecA*). The PCR for amplification of *mecA* gene was performed in a total reaction volume of 20 µl for one sample. The sequence of primer used for amplification of *mecA* gene forward 5'-AAAATCGATGGTAAAGGTTGGC-3', Reverse 5'-CGTGTTCATCAACATTTAATGCAA-3' the band size (500pb). The PCR cycling protocol was applied as following: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 second, annealing at 55°C for 30 second and extension at 72°C for 30 second, followed by a final extension at 72°C for 10 min. Agarose Gel Electrophoresis and Visualization of PCR Products: 5 µl of each amplicon was electrophoresed in 2 % agarose gel.

Results and Discussion:-

A total number of 57 isolates were obtained from 84 samples collected from clinical sources included wound swab, burn swab, ear swab and urine. All samples were cultured on blood agar and incubated at 37°C for 24 hours. According to the preliminary results, table [1] showed the ratio of sample contain *mecA* gene of *S. epidermidis* from clinical sources.

Table 1:- The sample collection and ratio of *mecA* gene contain.

Sample source	Ratio of sample collected	Ratio of <i>mecA</i>
wound	42	34
Ear swab	30	15
Urine	9	8
Burn	3	0
Total	84	57%

The results it is reported that from (105) clinical sample isolates (84) isolates were *Staphylococcus epidermidis*. Several biochemical tests were carried out to identify the *S. epidermidis*. All Gram-positive isolates gave positive results in the Catalase tests. The positive reaction indicated the liberation of free oxygen as gas bubbles after mixing of hydrogen peroxide solution with a little amount of bacterial growth [19]. In order to support the previous biochemical test, coagulase-negative and detect the ability produce of biofilm by growth on Congo-red agar biofilm producers form black colonies on CRA, whereas non-producers form red colonies [20]. The results revealed that from all 84 isolates, it was obtained 62 (73.8%) showed resistant to the Methicillin while 22 (26.2%) were sensitive to the Methicillin (inhibition zone of 23mm or less by Kirby-Bauer's disc diffusion method using 5µg methicillin disc on Mueller-Hinton agar). This result agreed with the study by (Martinet *et al.*, 2013) which found 73.8% of *S. epidermidis* isolates were resistant to methicillin by disc diffusion method [21].

PCR results indicated that seventy five 57(61.2%) isolates were positive for *mecA* genes as indicated by 500 bp

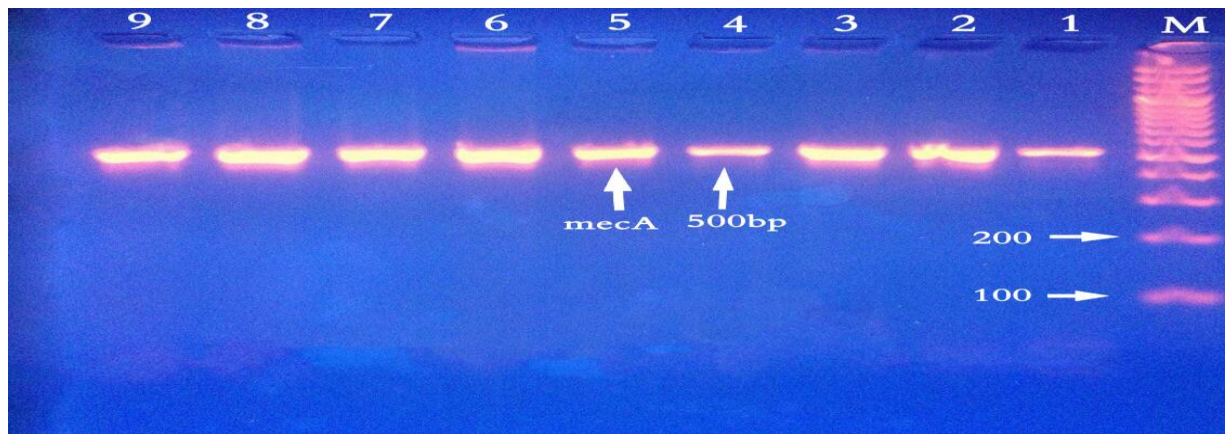


Figure 1:- Agarose gel electrophoresis (2% agarose, 5 V/cm²) PCR results with primer for *mecA* gene. M: Molecular size marker; lane 1-9 (500 bp) band obtained with DNA from *mecA* gene.

Whereas 27 (38.8%) *mecA* gene negative. The result that obtain by PCR of the of *S. epidermidis* isolate agree with our study by (Contreras *et al.*, 2013) it was found 64% of the *S. epidermidis* isolated resistance to methicillin and carried the *mecA* gene [22]. Genotypic methods are more accurate in detecting methicillin resistant *Staphylococci* as compared to conventional susceptibility methods. In addition, several culture conditions can also influence methicillin resistance such as the temperature, pH and concentration of NaCl in the medium [23]. Strains with low resistance level are usually complicated by these factors and impair the process of detection. The greatest disadvantage of phenotypic methods is that it is time-consuming [24]. And sometimes difficult to detect some strains; PCR can produce results within 24 hours as compared to the conventional methods which requires at least 48 hours there for the molecular detection methods are more preferred, favorable and accurate than the phenotypic method [25]. The study results indicate that 61.2% of the 57 methicillin-resistant strains of *S. epidermidis* samples were *mecA* gene positive. Whereas 27(38.8) % samples as *mecA* negative by PCR method. However, the reason for the of the *mecA* negativity can be attributed to one of the following:

1. Differing levels of *mecA* gene expression of methicillin resistance, occurring every 104 or 106 cells [26].
2. Absence of penicillinase plasmid, which otherwise plays an important role in the stability and phenotypic expression of the *mecA* gene [27].

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