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STUDY THE COAGULASE GENE IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DIFFERENT SOURCES BY USING PCR AMPLIFICATION

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ABSTRACT
Coagulase enzyme is one of the virulence factors of *Staphylococcus aureus* . This study is based on the molecular analysis of the coagulase encoding gene(*coa*). The polymorphism of the *coa* gene existing in different *S.aureus* have been analyzed in this study. Twenty-four isolates (24) of *S. aureus* were obtained from ninety-five samples (95) which were collected three hospitals in Baghdad and the General Health Lab. These isolates were collected from different sources (patients, food and drinking water) .They were grouped according to their sources. The clinical samples included, wound swab, burn swab, ear swab, urine and blood. The food samples included poultry meat and cheese.DNA from each sample was isolated and the *coa* gene was amplified with appropriate primers. The PCR results of 24 coagulase positive *S.aureus* gave three different size of amplicons variation among different *S.aureus* isolates of samples, which may be considered as an important criterion while treating *Staphylococcal* infections or source.

KEYWORD: *Staphylococcus aureus, coagulase gene, polymorphism.*

INTRODUCTION
Staphylococcus aureus is important pathogenic bacteria responsible for a range of diseases in humans. Hospital-acquired infections and antibiotic-resistant strains attributed to this bacteria have become endemic in hospitals in many countries and are associated with serious public health issues. The new strains cause severe community-acquired infection in immuno-compromised patients, as well as in healthy people.[1]

The clinical syndromes caused by this bacteria can be grouped as: coutaneous infections which include folliculitis, wound infections, toxin-mediated infections that include toxic shock syndrome, food poisoning, scalded skin syndrome which is seen in children under the age of four and other diseases such as pneumonia, bacteremia, endocarditis, osteomyelitis and septic arthritis.[2] Most of the species are coagulase-negative. However, some are coagulase-positive, such as *Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus delphini*.[3]

The *coa* gene amplification has been considered a simple and accurate method for typing of *S. aureus* isolated from distinct sources, the coagulase protein is an important virulence factor of *S. aureus*. The *coa* has a polymorphic repeat region that can be used for differentiating *S. aureus* isolates. The variable region of *coa* is comprised of 81bp tandem short sequence repeats (SSRs).[4] *The aim of this study was to detect the presence of coa gene in S. aureus from different sources by PCR amplification.*

MATERIAL AND METHOED.
Sample collection
Thirty-seven isolates were obtained from 95 samples collected from different sources (26 patient's isolates and 11 food isolates). All samples were cultured on blood agar and incubated at 37ºC for 24 hours. They were grouped according to their sources. The clinical samples included wound swab, burn swab, ear swab, urine and blood. The food samples included poultry meat and cheese.

All the isolates
were identified on the basis of colony morphology and positive coagulase tube test. Coagulase-positive staphylococcal isolates were confirmed as *S. aureus* using a commercial biotyping system (api STAPH, bioMerieux, Inc., Hazelwood, MO) (5,6,7).

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. aureus* strains isolated from different samples was extracted.

DNA extraction

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

coagulase gene Detection

Staphylococcus aureus. isolates were investigated for the presence of Coagulase gene (coA). The PCR for amplification of *coa* gene was performed in a total reaction volume of 25 µl for one sample. The sequence of primer used for amplification of *coa* gene forward CGA GAC CAA GAT TCA ACA AG , Reverse AAA GAA AAC CAC TCA CAT CA. The PCR cycling protocol was applied as following: initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 30 second, annealing at 58°C for 2 min and extension at 72°C for 4 min, followed by a final extension at 72°C for 7 min. Agarose Gel Electrophoresis and Visualization of PCR Products: 5 µ l of each amplicon was electrophoresed in 1.5 % agrose gel.[8]

RESULTS AND DISCUSSION

A total number of 37 isolates were obtained from 95 samples collected from different sources (26 patient's isolates and 11 food isolates). All samples were cultured on blood agar and incubated at 37ºC for 24 hours. They were grouped according to their sources. The clinical samples included wound swab, burn swab, ear swab, urine and blood. The food samples included poultry meat and cheese. According to the preliminary results, table [1] showed the isolation of different number of *S.aureus, S.epidermidis, S.haemolyticus, S.hominis and S.saprophyticus* from different sources, we ignored 38 samples (47.56%) as non-Staphylococcus samples. In this research from 26 clinical *Staphylococcus* spp. Isolates, 17 isolates were *Staphylococcus aureus* (65.38%) and CoNS where constituted (34.62%).

Table [1] Demographic distribution of isolates in terms of Sample origin

Source		**No: of samples**	**No: of Isolates**	***S. aureus***	***S. epidermidis***	***S. haemolyticus***	***S. hominis***	***S. saprophyticus***
clinical	Wound	10	5	4	1	0	0	0
	Burns	20	10	7	1	1	0	1
	Blood	10	4	2	0	2	0	0
	Urine	10	4	2	2	0	0	0
	Ear	10	3	2	1	0	0	0
Food	Poultry meat	20	7	4	1	1	1	0
	Cheese	15	4	3	0	0	1	0
Total		**95**	**37**	**24**	**6**	**4**	**2**	**1**

The clinical isolate results were close to Mousa[9] results. She reported that from (150) clinical *Staphylococcus* spp. isolates (100) isolates were *Staphylococcus aureus* which constituted (66.66%) and CoNS constituted (33.33%).

Several biochemical tests were carried out to identify the *S. aureus*.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[10] In order to support the previous biochemical test, DNase tests and tube coagulase tests were carried out. The combination of all the biochemical tests increased the sensitivity to identify the *S. aureus* among the bacterial isolates. The coagulase test tubes were carried out among the 24 MSA positive bacterial isolates of them all 24 isolates showed a positive coagulase test. The coagulase enzyme has the ability to coagulate the plasma in the coagulase tests tube all 24 tubes marked the clot formation.

DNase results were 20 (83.33%) and appeared positive and 3 isolates (12.5%) was negative DNase results, one food isolates (4.16%) had unclear results for thermonuclease product in cultural tests. Ismael[11] also had the same results in the coagulase test by getting (100%) positive coagulase results for 78 Staphylococcus *aureus* isolates.

DNase production was detected by culturing the isolates on DNase agar, DNase is an extracellular enzyme that cleaved DNA into subunits composed of nucleotides (Oligonucleotides). The appearance of clear zone around bacterial growth was considered as the positive activity that indicated the presence of Deoxyribonuclease enzyme hydrolyses DNA.[12]

All *Staphylococcus aureus* local isolates samples 24 produced PCR bands through (*Coa*) gene detection but these bands were in three molecular sizes ranging from 730 pb , 810 pb and 890 bp Figure [1]. The products 730 bp in size were the most frequent and accounted for (58.33%) of the isolates.

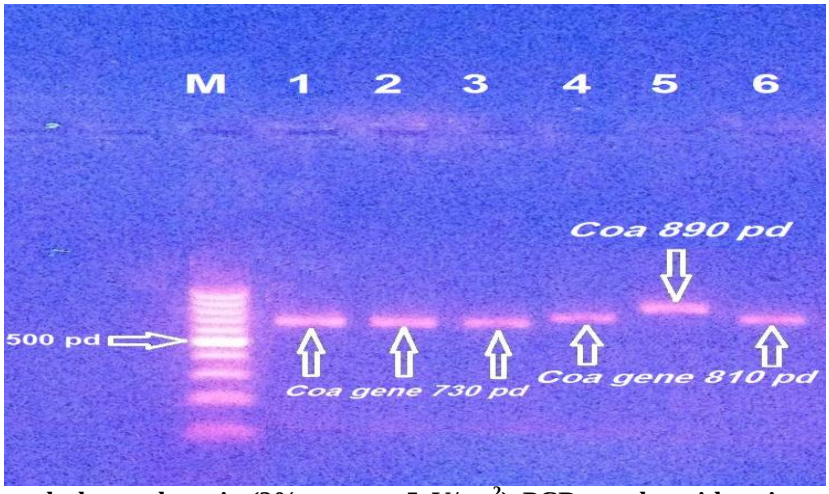

Figure (1): Agarose gel electrophoresis (2%agarose, 5 V/cm^2) PCR results with primer for *Coa* gene . M: Molecular size marker; lane 1,2,3 (730 bp), 4,6 (810 pb) 5 (890 pb) bands obtained with DNA from *Coa* gene.

Table: (2) Numbers of the three size of PCR product bands (730, 810 and 890 bp) of coagulase gene for Staphylococcus aureus according to their source.

Source		Number of *S. aureus*	730 pb	810 pb	890 pb
Clinical	Wound/ abscess	4	3	1	0
	Burns	7	4	2	1
	Blood	2	1	1	0
	Urine	2	1	1	0
	Ear	2	1	1	0
Food	Poultry meat	4	4	0	0
	Cheese	3	0	1	2
Total numbers		24	14	7	3

There was no amplification product of the DNA from *S. epidermidis, S. haemolyticus, S. hominis,* and *S. saprophyticus.* Reproducibility of the PCR products was demonstrated with 100% of the tested isolates, if we compare between the phenotype and genotype coagulase detections results we will find them identical (100%).

According to the results mentioned in table [2], the apparent of 2 (60%) bands with (890pb) in length from total 3 bands with contaminant cheese and one of that type of bands (890 pb) in all clinical isolates. This It may mean that the type of bands (890 pb) as a detection for Coagulase (*Coa*) gene in *S. aureus* DNA is related to the sources of the bacteria.

Although[13] mentioned in their research that after detecting for (Coa) gene in *S. aureus* isolated DNA, the product of the PCR was five different bands in size (730, 810, 890, 970 and 1050 pb) by using the same forward and reveres primers. While[14] found the coagulase gene typing. PCR products of 9 sizes, ranging from 81 to 1215 bp in increments of 81 bp (81, 243, 405, 486, 729, 810, 891, 972, and 1215 bp), were obtained. Pattern electrophoresis analysis generated 3 different types (Co1, Co2, and Co3) and 10 subtypes of band patterns. The amplification of DNA of S. aureus isolates obtained from human and animal sources revealed four amplicons (723, 812, 648 and 913 bp), These amplicons could classify the isolates into 4 groups (human) or 5groups (animal)[15] Similar results were obtained by Himabindu *et al*.[16] who showed that the sizes of PCR products obtained after amplification of *S.aureus* of human subjects rang from 650-1000 bps.

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