



Molecular Typing of *Staphylococcus aureus* by DNA Restriction Fragment Length Polymorphism of *coa* Gene

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Abstract: The aim of this study was to determine the genotypic characteristics of *S. aureus* isolates by using coagulase gene typing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of coagulase gene. One hundred and seventy two wound swab specimens were collected from patients in AL-Kindy Teaching Hospital, Al-Wasity Hospital, Baghdad Al-Taalemy Hospital and Al Shaheed Gazi Hospital in Baghdad during the period from September 2013 to January 2014. All isolates were diagnosed depending on microscopical and biochemical tests. It was found that 47(27.33%) isolates were identified as *S.aureus*. The amplification of the *coa* gene of *S.aureus* strains generated 5 different genotypes: I, II, III, IV, V based on different size of polymorphism ranging from 500-900bp. The majority (44.7%) of the strains were of *coa* gene type II. PCR-RFLP of *coa* gene exhibited 11 patterns which were obtained with *AluI* digests of PCR products. The number of fragments varied from one to three with sizes of the fragments varied from 80 to 700 bp. Type IX was the most common and accounted for 27.7% of the isolates. PCR-RFLP analysis, showed discriminatory power, reproducibility, easy interpretation and can be considered as a promising alternative for the epidemiological typing of *S.aureus* isolates.

Keywords: *Staphylococcus aureus*, molecular typing, *Coa* gene, PCR- RFLP.

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Introduction

Staphylococcus aureus is one of the important pathogens in many countries causing infection in the hospitals (1). It can cause a wide range of diseases ranging from minor skin infections to more life-threatening conditions such as osteomyelitis, bacteraemia and infective endocarditis (2).

Typing is an important tool for infectious disease outbreak investigations where the aim is to define a local and temporal increase in the

incidence of infection by a certain bacterial species. The typing of outbreak strains facilitates the development of outbreak control strategies, defining of the extent of epidemic spread of bacterial clones and the number of clones involved in the transmission and infection, monitoring of the reservoirs of epidemic clones, and control the evaluations of the efficacy of control measures, such as monitoring vaccine efficacy (3,4).

A variety of molecular methods have been developed for epidemiologic

typing of *S.aureus* strains. Among these methods polymerase chain reaction (PCR) of the coagulase (*coa*) gene and polymerase chain reaction restriction fragment length polymorphism of the *coa* gene (PCR-RFLP) (5,6).

coa gene typing is an attractive method for clinical laboratories because of its ease and speed (7). Its discriminatory power relies on the heterogeneous region which contains 81-base pairs tandem repeats at the 3' ends (8). PCR amplifications of these particular regions produce DNA fragments of different sizes and are highly polymorphic with regard to the number and sequence of the repeats (9). PCR products of the *coa* gene can be further discriminated through digestion with *AluI* enzyme (10).

The aim of this study was to investigate epidemiologic typing of *S.aureus* strains by using coagulase gene typing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of coagulase gene.

Materials and Methods

Sample Collection and Bacterial Identification

One hundred and seventy two specimens were collected from four hospitals in Baghdad: AL-Kindi hospital, Al-Wasity hospital, Baghdad Al-Taalemey hospital and Al Shaheed Gazi hospital in the period between September 2013 and January 2014. The specimens included wound swabs. The isolates were cultured on the blood agar and identified by conventional methods including colony morphology, haemolysis, Microscopic examination

(gram stain) and biochemical tests including Oxidase, Catalase, Coagulase, Nitrate reduction, DNase and Urease tests. All the isolates were confirmed as *S.aureus* by API Staph system (11).

DNA Extraction

The bacterial genomic DNA was extracted from *S.aureus* isolates using Wizard® genomic DNA purification kit (promega,USA) according to the manufacturers protocol. DNA was electrophoresed in a 1% agarose gel.

PCR Amplification

Amplification of the 3' end of the *coa* gene was determined using the primers described by Janwithayanuchit *et al.* (9) 5'-ATA GAG ATG CTG GTA CAG G3' and 5'-GCT TCC GAT TGT TCG ATG C 3'. The total reaction volume was 50µl per-tube, containing 2.5unit of Taq DNA polymerase enzyme, 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 10 Pmol of each primer and 5ng DNA of each isolates. It was carried out in a thermal cycle (Thermo,USA) with the following programmed : initial denaturation at 94 °C for 3 min, followed by 30cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The PCR products were run on 2% agarose gel electrophoresis is followed by staining in ethidium bromide and visualized under UV transilluminator then compared with DNA ladder (100bp).

Restriction Fragment Length Polymorphism (RFLP) of Coagulase Gene

Ten of microliters of PCR product was digested with 2 units of restriction endonuclease *AluI* (Bioneer) at 37°C for 90 min. according to Janwithayanuchit *et al.* (9). The restriction digested fragments were run on 2% agarose gels, which were then stained with ethidium bromide and visualized under UV light.

Results and Discussion

Among 172 specimens from wound swabs, 47 isolate were identified as *S.aureus* according to the results of phenotypical assays. PCR amplification of the *coa* gene from 47 isolates generated five different type based on sizes, ranging from 500-900 bp were recognized as follows: *coa* type I (500bp), *coa* type II (600bp), *coa* type III (700bp), *coa* type IV(800bp) and *coa* typeV(900bp) (figure-1) and were found at 12.8%, 44.7% , 21.3%, 14.9% and 6.4%, respectively. The majority of the isolates exhibit *coa* gene type II with 600bp in size. The Predominance of a particular strain of *S.aureus* might be the result of its increased resistance to the host immune response compared to those with the rare genotypes which could have a lower resistance (12).

The result was compatible with the studies of Janwithayanuchit *et al.* and Talebi-Satluo *et al.* (9,10) using the same primer. They obtained four different *coa* gene types, wherese Sanjiv *et al.* (13) who carried out *coa* gene typing using similar primer found three different *coa* gene type. The reason for this polymorphism in the *coa* gene

among *S.aureus* isolates could be due to this gene which consists of three distinct regions: (i) the N-terminus containing the prothrombin-binding site, (ii) a central region which is highly conserved, and (iii) a C-terminal region composed of 81-bp tandem repeated units, which each encode 27-amino acid residues (9). The C terminal repeated units comprised four, five, six, seven and eight units of the 81-tandem repeat (14). Therefore, the size of 3' region of the *coa* gene is variable in *S.aureus* strains (15). However, it should be noted that the repeated units are not necessary for prothrombin binding or plasma clotting because of the lack of importance of the repeated units to this enzymatic function. Point mutations may occur without a consequence to the virulence potential of the strains (16,17).As summarized in the (Table-1). *AluI* restriction enzyme digestion of the PCR products generated 11 different *AluI* restriction patterns with the number of fragments varying from one to three and with sizes of the fragments varied from 80 to 700 bp (figure-2). Type IX was the most common and it accounted for 27.7% of the isolates.

The result was agreed with the study done by Esan *et al.* (1) who reported 11 RFLP patterns. Other researchers such as Goh *et al.* (17) reported 19 RFLP patterns. In contrast, Janwithayanuchit *et al.*,(9) reported only 4 RFLP patterns. As indicated above, the variations among RFLP of the coagulase gene may be due to the variation in the sequence of coagulase gene among different isolates leading to different restriction sites (18) .

When amplified *coa* gene was digested with *AluI* enzyme, eight strains were not

digested by the enzyme, This could be due to the lack of restriction sites for the enzyme in the variable region of the gene in these isolates which could happen due to the point mutations in the

repeated region of the *coa* gene abolishing the *AluI* restriction site (17) .

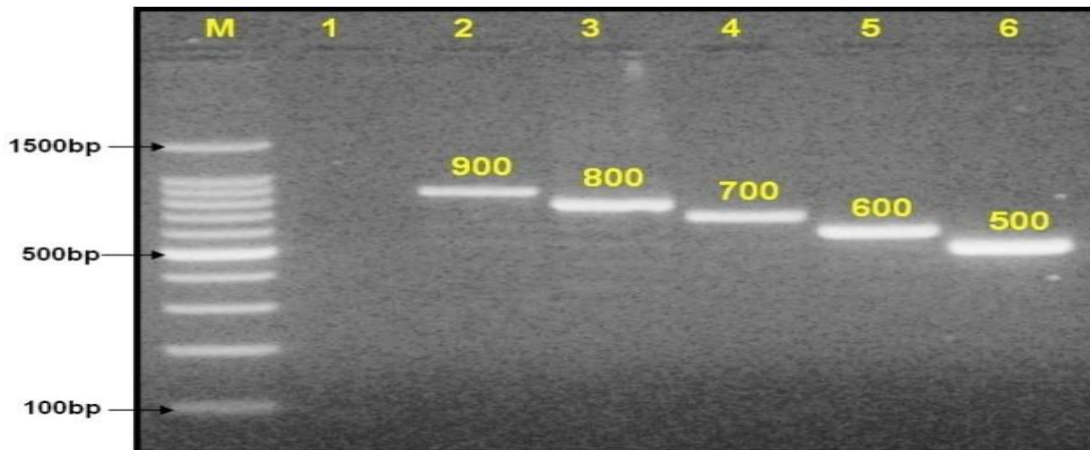


Figure (1): Agarose gelelectrophoresis of *coa* PCR products of *S.aureus* isolates. (M: 100 bp molecular weight marker, 2% agarose, TBE buffer (1X), 80V, 90 min; lane 1: negative control, lane 2: *Coa* gene type V (900 bp), lane 3: *Coa* gene type IV (800 bp) , lane 4: *Coa* gene type III (700 bp) , lane 5: *Coa* gene type II(600 bp), lane 6: *Coa* gene typeI(500 bp).

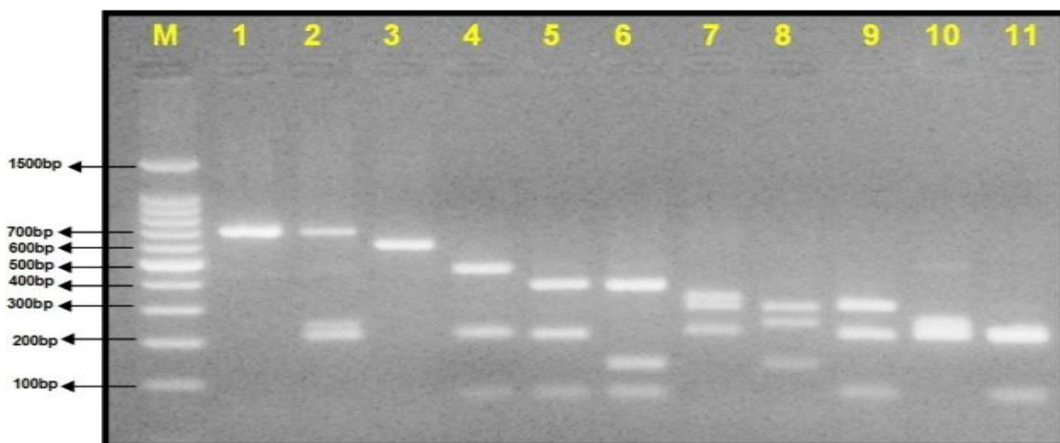


Figure (2): Agarose gel electrophoresis of *AluI* restriction fragments of PCR products (M: 100 bp molecular weight marker, 2% agarose, TBE buffer (1X), 80V, 90 min; Lanes 1-11: RFLP patterns obtained of coagulase PCR products with *AluI* endonuclease. Lane 1:TypeI (fragments 700 bp), Lane 2: Type II (fragments: 220-240 -700), Line 3: Type III (600 bp), Line 4: Type IV (fragments: 80-220-500), Line 5: Type V (fragments: 80-220-400 bp), Line 6: Type VI (fragments: 80-150-400 bp). Line 7: Type VII (fragments: 220-300-320 bp) ,Line 8: Type VIII (fragments: 150-240-300 bp), Line 9: Type IX (fragments: 80-220-300bp), Lane 10: Type X (fragments: 220-240), Lane 11: Type XI (fragments: 80- 220bp).

Table (1): RFLP patterns of *coa* gene

PCR-RFLP typing	Size of <i>AluI</i> fragments (bp)	<i>Coa</i> PCR product (bp)	NO.of isolates(%)
type I	700	700	2 (4.3)
type II	220-240-700	700	1(2.1)
type III	600	600	6 (12.8)
type IV	80-220-500	800	7(14.9)
type V	80-220-400	700	2 (4.3)
type VI	80-150-400	600	1(2.1)
type VII	220-300-320	900	3 (6.4)
type VIII	150-240-300	700	2 (4.3)
Type IX	80-220-300	600	13 (27.7)
Type X	220-240	700	3 (6.4)
Type XI	80-220	500 + 600	7(14.9)

In the present investigation different *coa* gene products and their restriction fragment length polymorphism for *S. aureus* isolates indicated great genotypic variability among the organisms. It was concluded that RFLP of the *coa* gene offers a good discriminatory power in typing *S. aureus* isolates.

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