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Genotyping of *Staphylococcus aureus* Isolates Based on Methicillin-Resistance Genes and its Relatedness to some Putative Virulence Factors

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Abstract
The emergence and spread of methicillin-resistant *Staphylococcus aureus (*MRSA) was a public health problem worldwide that causes nosocomial and community infections. Forty three isolates (71.66%) were characterized as *S.aureus*, were isolated from 60 different clinical specimens (blood, nose, wound, urine and vaginal) collected from patients from different hospitals of Baghdad. All isolates were resistant (100%) to Aztreonam, Carbenicillin, Cifixime, Cefoxitin, Ceftazidime, and showed high resistance to each of Methicillin, Oxacillin, Ampicillin and Penicillin . the MRSA isolates were typed based on (*SCC*mec) typing ,*the result revealed that SCC*mecIVa was the most common in isolates (41.86%), following type IVc (20.93%), type II(16.27%). Virulence factors were identified to detect genes encoding for Hlg,Pvl,ClfA,Tsst-1 and Eta, and the result showed the most prevalent gene was *hlg* (65.11%), following *pvl* (53.48%), *clfA* (51.16%), *tsst-1*(18.60%),*eta* (11.62%). The virulence genes profiles were observed, and the most frequent was *clfA-hlg-pvl* (23.25%), *clfA-hlg-pvl-tsst-1* (6.97%),*clfA-hlg-tsst-1*(4.65%).Analysis of genetic similarity relationship, showed the isolates of *S.aureus* were classified into two main clusters . This result indicates that there a diversity in virulence genes profiles among MRSA isolates according to *SCC*mec types, and SCCmec IVa carried *hlg, pvl , clfA* genes was the most prevalent in Baghdad hospital isolates .

Key words: MRSA, *SCC*mec, virulence genes profiles

1.Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a significant human pathogen. For many years it has been a common cause of nosocomial infections, and variants capable of causing infections in the community [community-acquired MRSA (CA-MRSA)] are an emerging and serious public-health issue (Chambers, 2001).

The development of resistance to a wide range of antibiotics in *S. aureus* is diversified, such as resistance to methicillin that takes the account of *S.aureus* to most β-lactams, macrolides and aminoglycosides (Kennedy *et al*., 2008). Resistance of *Staphylococcus aureus* to β-lactam antibiotics is associated with the expression of penicillin-binding protein 2a (PBP2a), which encoded by the *mecA* gene, which is located on a mobile genetic element, staphylococcal cassette chromosome mec (*SCC*mec) (Ito *et al*., 2004). *SCC*mec is a mobile genetic element characterized by the presence two essential genetic components (the *mec* gene complex and the *ccr* gene complex), and the junkyard (J) regions (Ma *et al*., 2002 ; Ito *et al*., 2004). The *mec* gene complex is composed of IS*431* and IS*1272*, *mecA*, and regulatory genes *mecR1* (encoding the signal transducer protein MecR1) and *mecI* (encoding the repressor protein MecI) (Ito *et al*., 2001 ; Kondo *et al*., 2007). The *ccr* gene complex (*ccrA*, *ccrB*, and *ccrC*) encodes recombinases of the invertase/resolvase family, which mediate the integration of SCCmec into and its excision from the recipient chromosome and are responsible for the mobility of this element. The rest of the SCCmec element is comprised of J regions (J1, J2, and J3) that are located between and around the *mec* and *ccr* gene complexes and contain various genes or pseudogenes, including plasmid- or transposon-mediated resistance genes for non-β-lactam antibiotics (Ito *et al*., 2003). To date, there are five classes (A, B, C1, C2, and D) of *mec* gene complexes and five allotypes (types 1, 2, 3, 4, and 5) of *ccr* gene complexes (Hiramatsu *et al*., 2001). combinations of these complex classes and allotypes generate various SCCmec types. SCCmec elements are currently classified based on the nature of the *mec* and *ccr* gene complexes into types I (combination of the type 1 *ccr* and the class B *mec* gene complex; 1B), II (2A), III (3A), IV (2B), V (5C2), and VI (4B), , and are further classified into subtypes according to differences in their J region DNA(Ito *et al*., 2004), type VII (5C1) (Berglund *et al*., 2008).and type VIII(4A)(Zhang *et al*., 2009). *Staphylococcus aureus* is a pathogen with the capability to produce a series of virulence factors that contribute to the severity of infections. These factors include microbial surface components that recognize adhesive matrix molecules (MSCRAMM), cytolytic toxins, exoenzymes, exotoxins, hemolysins, leukocidins (such as PVL), and superantigens. The group of superantigens includes staphylococcal enterotoxins (SE), toxic shock syndrome

toxin (TSST), and exfoliative toxins. These exoenzymes and exotoxins demonstrate proteolytic activity and toxic or lytic effects in the cells, facilitating local invasion and dissemination (Dinges *et al*., 2000). The goals of this study was to investigate the molecular characteristics of *SCC*mec typing of MRSA isolates and relatedness with their virulence genes in Baghdad hospitals.

2.Materials and Methods

2.1 Bacterial isolates

A total of 60 *S.aureus* isolates were obtained from different clinical specimens such as urine, wound, blood, nose and vagina, which were collected from different local hospitals in Baghdad. The isolates were identified as *S. aureus* using custom tests (Gram's stain, ferment mannitol, catalase, oxidase and free coagulase tests)(William *et al* .,2009).

2.2 Antimicrobial susceptibility tests

The antimicrobial susceptibility pattern was determined by the disc diffusion test against 19 antimicrobial agents (Amikacin, Amoxicillin/ clavulanic acid, Ampicillin, Aztreonam, Carbenicillin, Ceftazidime, Cifixime, Chloramphenical, Cefoxitin, Erythromycin, Gentamicin, Imipenem, Methicillin, Oxacillin, Penicillin G, Piperacillin, Rifampin, Tetracycline, Vancomycin). susceptibility to Antimicrobial were determined by using Kirby-Bauer method according to the standard guidelines recommended by National Committee for Clinical Laboratory Standards (NCCLS, 2007).

2.3 DNA extraction

Whole genomic DNA was isolated from *S.aureus* using the Exiprep ™ 16 plus Bacteria Genomic DNA kit (Bioneer /Koreae) and used as template in all PCR analysis.

2.4 PCR amplification

All PCR reactions were amplified in a thermal cycler (labnet-USA).The following components were used : 12.5 µl 0f Co Taq®Green Master Mix (Promega /USA), that contained Taq DNA polymerase, $MgCl_2$ deoxynucleosides (dNTP), buffer,1 µl of each primer (10pmol), 2µl of DNA template and sterile distilled water was added to achieve a total volume of 25 µl .

2.5 Detection of *nuc* and *mecA* genes

The thermostable nuclease gene (*nuc*) and *mecA* gene were amplified for the identification of *S.aureus* (*nuc* gene) and detection MRSA (*mecA* gene). The Oligonucleotide primers for *nuc* gene were designed based on published nucleotide sequence (Brakstad *et al.,* 1992), the sequence of primer: *nuc*-F 5'-GCGATTGATGGTGATACGGTT-3' and *nuc*-R 5'-AGCCAAGCCTTGACGAACTAAAGC-3'.While primer for *mecA* gene were designed according to Zhang *et al*., (2005), the sequence of primer: *mec*A –F '5'- GTG AAG ATA TAC CAA GTG ATT -3' and *mec*A-R 5'- ATG CGC TAT AGA TTG AAA GGA T -3' . The amplification of the *nuc* gene was performed in 37 cycles, DNA denaturation at 94°C for 1 min, primer annealing at 60°C for 45 sec, and DNA extension at 72°C for 1.5 min. While the *mecA* gene was performed in 30 cycles, DNA denaturation at 94°C for 1 min, primer annealing at 54°C for 45 sec, and DNA extension at 72°C for 2min. PCR products were checked by agarose (2% w/v) gel electrophoresis at 70 V for 1.5 hr .

*2.6 SCC*mec typing

*SCC*mec typing of the MRSA isolates was performed with specific primers were designed according to Zhang *et al*., (2005), the primers and their sequences are listed in Table (1). The amplification was performed under conditions in 30 cycles , DNA denaturation at 94°C for 1 min, primer annealing at 56°C for 45 sec, and DNA extension at 72°C for 2 min, after amplification All PCR products were analyzed through agarose gel electrophoresis on 2% agarose gels at 70 V for 1.5 hr

2.7 Detection of Virulence genes

Sequences specific for *hlg, pvl, clfA, tsst-1, eta,* encoding Hlg, PVL,CLFA, TSST-1and ETA, respectively, were detected by PCR with the primers shown in Table (2). Amplification of the virulence genes was carried out under following conditions in 30 cycles, DNA denaturation at 94°C for 1 min, primer annealing at 60-70°C for 45 sec, and DNA extension at 72°C for 2 min, the PCR products were analyzed through agarose gel electrophoresis on 2% agarose gels at 70 V for 1.5 hr.

Table 1 . Oligonucleotide primers sequences and PCR product for *SCC*mec typing.

Primer	Sequence (5'----------3')	PCR product
Type I-F	GCTTTAAAGAGTGTCGTTACAGG	613bp
Type I-R	GTTCTCTCATAGTATGACGTCC	
Type II-F	CCATATTGTGTACGATGCG	398bp
Type II-R	CGAAATCAATGGTTAATGGACC	
Type III-F	CCATATTGTGTACGATGCG	280bp
Type III-R	CCTTAGTTGTCGTAACAGATCG	
Type IVa-F	GCCTTATTCGAAGAAACCG	776bp
Type IVa-R	CTACTCTTCTGAAAAGCGTCG	
Type IVb-F	TCTGGAATTACTTCAGCTGC	493bp
Type IVb-R	AAACAATATTGCTCTCCCTC	
Type IVc-F	ACAATATTTGTATTATCGGAGAGC	200bp
Type IVc-R	TTGGTATGAGGTATTGCTGG	
Type IVd-F	CTCAAAATACGGACCCCAATACA	881bp
Type IVd-R	TGCTCCAGTAATTGCTAAAG	
Type V-F	GAACATTGTTACTTAAATGAGCG	325bp
Type V-R	TGAAAGTTGTACCCTTGACACC	

Table 2. Oligonucleotide primers sequences and PCR product for virulence genes.

Primer	Sequence (5'----------3')	PCR product	Reference
Pvl – F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433pb	Jarraud *et al* ., 2002
Pvl – R	GCATCAAGTGTATTGGATAGCAAAAGC		
Hlg –F	GTCAYAGAGTCCATAATGCATTTAA	535pb	
Hlg – R	CACCAAATGTATAGCCTAAAGTG		
ETA – F	ACTGTAGGAGCTAGTGCATTTGT	190pb	
ETA – R	TGGATACTTTTGTCTATCTTTTTCATCAAC		
TSST-F	GCT TGC GAC AAC TGC TAC AG	559pb	Monday and Bohch,1999
TSST-R	TGG ATC CGT CAT TCA TTG TTA T		
ClfA – F	ATTGGCGTGGCTTCAGTGCT	292pb	Tristan *et al* ., 2003
ClfA –R	CGTTTCTTCCGTAGTTGCATTTG		

2.8 Data Analysis

The similarity between the *S. aureus* isolates was determined on the basis of the Jaccard similarity using Unweighted Pair-Group Method with Arithmetic Average (UPGMA) (Rohlf, 1998).

3 Results and Discussion

3.1 Identification of isolates

Sixty clinical samples collected from different sites of patients such as (urine, wound , blood, nose and vaginal) in Baghdad hospitals, 43 isolates were identified as *S. aureus* were characterized according to Bergey's manual of Systematic Bacteriology (Wiliam *et al*., 2009). blood samples was represented the high percentage of *S.aureus* (90%) following nose (77.77%), wound (71.42%), urine (66.66%) and (60%) vaginal. 43 isolates gave positive results for catalase and coagulase production tests, the ability to ferment mannitol aerobically, and negative for oxidase test, and all of *S. aureus* isolates were positive to *nuc* gene .

3.2 Antimicrobial susceptibility

The fourty three isolates of *S.aureus* were found to be resistant (100%) to Aztreonam, Carbenicillin, Cifixime, Cefoxitin and Ceftazidime, and all these isolates were susceptible (100%) to Piperacillin, Chloramphenical, Rifampin, Imipenem, Vancomycin. The rates to others antimicrobial agents tested were 93.03% to each of Methicillin, Oxacillin, Ampicillin, Penicillin, 72.09% to Gentamicin, 48.83%, to Amikacin, 39.53% to Erythromycin, 34.88% to 34.88% to Amoxicillin/ clavulanic acid, 11.62% to Tetracycline (figure 1).

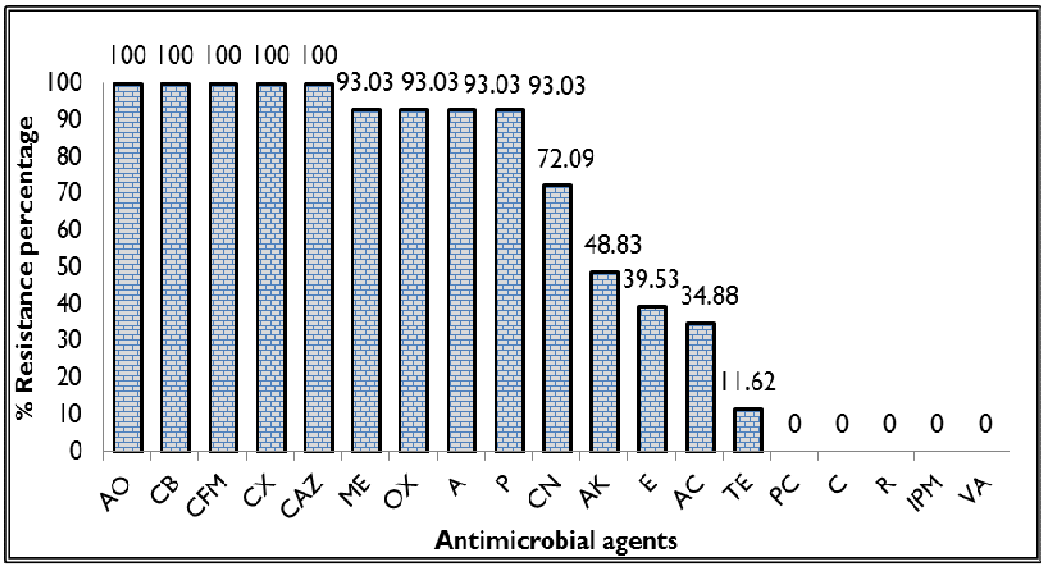

Figure 1. Susceptibility of *S. aureus* isolates to antimicrobial agents.

3.3 Identification of MRSA

Among 43 isolates of *S. aureus*, 37 isolates (86.04%) were MRSA (*mecA* positive) while 6 isolates (13.95%) were MSSA (*mecA* negative). Results revealed that 37 isolates that gave positive results for *mecA* gene showed resistance to methicillin in antimicrobial susceptibility test and carried *mecA* gene while among these isolates 6 of them gave negative results for *mecA* gene , 3 isolates were susceptible to methicillin and did not carry *mecA* ,whereas other 3 isolates showed high- level resistance to methicillin in antimicrobial susceptibility test but lacked to *mecA* . These results were in agreement with results reported by Nakaminami *et al*., (2008) , who showed that among the MRSA, two strains showed resistance to oxacillin but lacked to *mecA* . Whereas Suhaili *et al*., (2009) noted that the results of *mecA* were in agreement with the disc diffusion test results . There is no optimal phenotypic method for detecting methicillin resistance in *S. aureus* (Baddour *et al* ., 2007).

3.4 Molecular *SCC*mec typing

*SCC*mec typing is one of the most important molecular tools available for understanding the epidemiology and clonal strain relatedness of MRSA, particularly with the emerging outbreaks of community- acquired MRSA occurring on a worldwide basis (Ito *et al* ., 2001). In this study , its determined the *SCC*mec types of the Baghdad hospitals isolates by using PCR technique targeted the unique and specific loci of *SCC*mec types and subtypes(I, II, III, IVa, IVb, IVc, IVd, and V. 37 isolates among total of 43 isolates were analyzed through

*SCC*mec typing , whereas 6 isolates were not typeable among five *SCC*mec typese. *SCC*mec typing results showed that the most common was type IV which was found in 28 isolates (65.11%). Among the 28 type IV isolates, three *SCC*mec subtypes were found ,IVa, IVc, IVd, the most common subtypes was type IVa (41.86%) following type IVc (20.93%),type IVd (2.32%) .Whereas subtype IVb did not found in 28 isolates (figure 2). These results were in agreement with pervious study by Geng *et al* ., (2010) reported the most common was type IV (67.7%) subtype IVa (77.6%) followed type IVc (11.9%) in Chine isolates. Whereas Machuca *et al* ., (2013) noted that the most common was type IV and subtype IVc (75%), subtype IVa (4%) in MRSA isolates from pediatric patients in Colombia . While type II was found in 7 isolates (16.27%), this result was disagreement with result of Ahmad *et al* ., (2009) found that none of isolates in Malaysia carried *SCC*mec type II . The type III was found in one isolate (2.32%), these results were disagreement with Wang *et al* .,(2013) noted that the most MRSA isolates with Blood stream infections in China was *SCC*mec type III(84.6%) and Ahmad *et al* ., (2009) reported that the most common was *SCCmec type* III (96.81%) in Malaysia hospitals. Whereas type V was found in one isolate (2.32%),but other previous study by Geng *et al* ., 2010 found 32.3% type V in China isolates and Nakaminami *et al* ., (2008) found 7.2% typeV in Japan isolates. The results of *SCC*mec types revealed no isolates related to *SCC*mec type I. *SCC*mec types I to III are larger elements (34 to 67 kb), and contain resistance determinants in addition to *mecA*, and are more frequently found in HA-MRSA(Ito *et al*., 2001) In contrast, the *SCC*mec types IV- V element, which is the smallest of the *SCC*mec elements (21 to 24 kb) and usually devoid to resistance determinants other than *mecA* and frequently found in CA-MRSA (Okuma *et al* ., 2002) . From these results , revealed there was a diversity in *SCC*mec types from those of other countries, according to geographic location and clinical-epidemiological factors, and the *SCC*mec type IV was the most prevalent among *SCC*mec types in worldwide, due to the small size of *SCCmec* type IV which it easily transferred to other isolates of *S.aureus* .

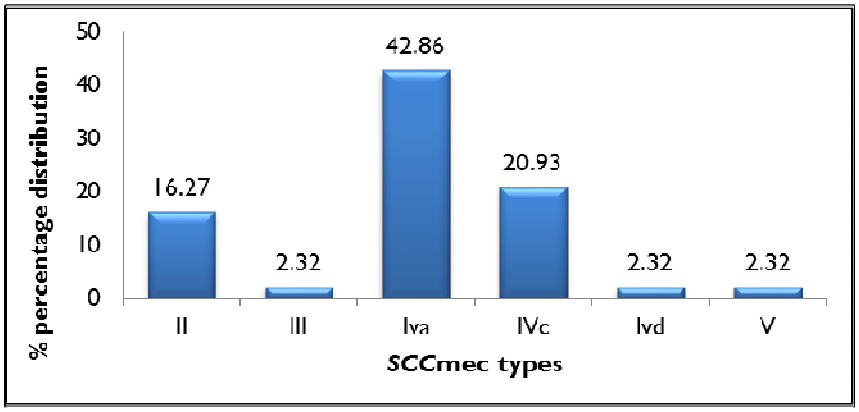

Figure 2. :Percentage distribution of *SCC*mec types among MRSA isolates.

3.5 Virulence genes profiles

The 35 isolates among 43 isolates were positive at least to one of virulence genes ,whereas 8 isolates did not produce any virulence genes. The most prevalent virulence genes detected were *hlg* (65.11%), *pvl* (53.48%), *clfA* (51.16%), *tsst-1* (18.60%) and *eta* (11.62%) (figure 3). The isolates of *S. aureus* produces a large number of toxins including hemolysins, the PVL (lukF/S-PV) ,Staphylococcal enterotoxins (SEs), exfoliative toxins (ETA and ETB) and the toxin of toxic shock syndrome-1 (TSST- 1) (Larkin *et al*., 2009).In this study, the majority of MRSA isolates were found to harbor the *hlg, pvl* and *tsst-1*gene and only a minority were *eta* gene. Some isolates produced multiple virulence genes, the most prevalent were *clfA- hlg-pvl* (23.25%), *clfA- hlg-pvl-tsst-1* (6.97%),*clfA- hlg-tsst-1* (4.65%).This results was in agreement with previous study by Machuca *et al* ., (2013),who found The most common toxin genes detected were *hlg* (100%), *pvl*(88%), but differs in presence of *tsst-1* gene, which were not detect in any of the MRSA isolates from pediatric patients in Colombia. *S. aureus* expresses many surface proteins of the microbial surface components recognizing adhesive matrix molecules family (MSCRAMM), which specifically recognize and bind to the extracellular matrix components of the host. The results in this study indicate that the *clfA* gene encode clumping foctar A(clfA), presence in most of *S.aureus* isolates this result was in agreement with results of previous studies by Peacock *et al* ., (2002) and Machuca *et al* ., (2013) they confirmed that these genes carried by most of *S.aureus* isolates .The results of this study revealed that the distribution of virulence genes various with the type of infection and the isolate which causing it, in all different clinical samples of *S. aureus* , the *pvl* gene was present, this result agree with study by Jimenez *et al* .,(2011) noted the virulence genes were diverse among types of infection and the *pvl* gene

presented in all MRSA infection, and the results showed the *hlg, clfA, tsst-1* genes were present in blood, wound, urine, vaginal samples, but not prevalent in nasal samples. While The prevalence of the ET encode *eta* gene in *S. aureus* was low, this result was coincides with the results of other investigation even through epidemiological data on *S.aureus* isolates (Gemmell, 1995), and agreement with previous studies of Jarraud *et al* .,(2002), Becker *et al* ., (2003), they found low rates of *eta* gene detected in *S.aureus* isolates , in the present study, *eta* gene was detected only in isolates from wound (3 in MSSA isolates, 2 in MRSA), the exfoliative toxins (ETs) was caused impetigo consider one of the major bacterial infections (Plano, 2004), this agreement with Nakaminami *et al* ., (2008) found that *eta* gene was primarily in isolates without *mecA* gene .

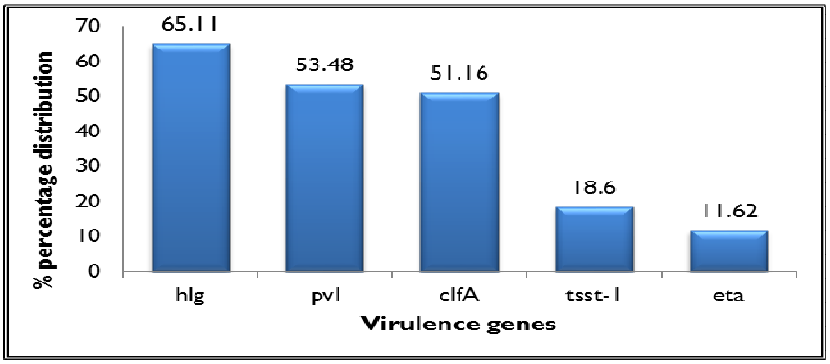

Figure 3. Percentage distribution of virulence genes among MRSA isolates.

3.6 Relationship between *SCC*mec types and virulence genes

Some virulence gene was associated with *SCC*mec type, *hlg* gene was associated with III, IVa, IVc,V *SCCmec* types, but more frequently in *SCCmec* IVa (94.44%) .The *pvl* gene was associated with *SCCmec* II, IVa, IVc, V, but the most prevalent in *SCC*mec types IVc and IVa (77.77%) .The *clfA* gene was associated with *SCC*mec II, III, IVa, IVc , V, but more frequently in *SCCmec* IVa (88.88%).The *tsst-1* gene was associated with IVa, IVc *SCC*mec types (22.22%).The *eta* gene was associated only with *SCC*mec IVa (table 3). These results were coincides with the results of study by Jimenez *et al* ., (2011) found the *pvl* gene was more prevalent in *SCC*mec IVc 94%, *SCC*mec IVa (100%) and *tsst-1* gene was detected in *SCC*mec types IVa, IVc, but disagreement with result of presence the *eta* gene ,they found *eta* gene was only present in *SCC*mec V among MRSA isolates in Colombia.

Table 3. Virulence genes distribution among *SCCmec* types of MRSA isolates

	SCCmec types					
Virulence Genes	II (n=7) n(%)	III (n=1) n(%)	IVa (n=18) n(%)	IVc (n=9) n(%)	IVd (n=1) n(%)	V (n=1) n(%)
Hlg	0(0)	1(100)	17(94.44)	4(44.44)	0(0)	1(100)
Pvl	1(14.28)	0(0)	14(77.77)	7(77.77)	0(0)	1(100)
clfA	1(14.28)	1(100)	16(88.88)	2(22.22)	0(0)	1(100)
tsst-1	0(0)	0(0)	4(22.22)	2(22.22)	0(0)	0(0)
Eta	0(0)	0(0)	2(11.11)	0(0)	0(0)	0(0)

The results of present study showed most of MRSA isolates carried multiple virulence genes and the most prevalent was *clfA- hlg-pvl*, *clfA- hlg-pvl-tsst-1*,*clfA-hlg-tsst-1*, and there a diversity in virulence genes profiles among *SCC*mec types isolates, the most *SCC*mec IVa isolates secreted more virulence genes, and most of *SCC*mec IVc isolates produced one virulence gene, only 3 isolates secreted multiple virulence genes, while the most of *SCC*mec II isolates did not produce virulence genes, only one isolate secreted *clfA, pvl* genes, and the *SCC*mec III isolate produced *clfA, hlg* genes and the isolate of *SCC*mec type V carried *hlg, pvl, clfA* genes. These results were consistent with study of Collins *et al* ., (2010) found the MRSA isolates carrying *SCCmec* II there was a reduction in virulence factor secretion, while isolates carrying SCC*mec* IV produced a more diverse range of factors (table 4). In this study we found that 41. 86 % of total isolates most commonly the *SCC*mec IVa , were carried *hlg ,pvl, clfA , tsst-1, eta*. Following *SCC*mec IVc isolates carried *hlg, pvl, clfA , tsst-1,*the one of *SCC*mec II isolate carried *pvl , clfA* genes, *SCC*mec III isolate carried *hlg , clfA* gene and *SCC*mec V isolate carried *hlg*, *pvl ,clfA* genes but *SCC*mec IVd isolate did not carry these genes. These results were in agreement with study reported by Abdel-Haq *et al*., (2009) in USA and Wu *et al*.,(2010) in China. Whereas the studies of Tokajian *et al*.,(2010) in Lebanon and Machuca *et al* ., (2013) in Colombia reported that most MRSA isolates were *SCC*mec IVc and carried *pvl* gene . These results indicated that the most MRSA isolates infection in Baghdad hospitals were molecular type *SCC*mec IVa which carried *hlg, pvl, clfA* genes and there great diversity of virulence genes profiles among MRSA isolates according to *SCC*mec types , in this study we confirmed that the *SCC*mec IVa carried *hlg, pvl, clfA* genes associated with infection in Baghdad hospitals.

Table 4 . Virulence genes profiles association with *SCC*mec types

Virulence genes profiles	***SCC*mec types**
clfA, hlg, tsst-1	IVa
clfA, hlg, tsst-1	IVa
clfA, hlg	IVa
clfA, hlg	III
clfA , hlg, pvl , tsst-1	IVa
clfA , hlg, pvl , tsst-1	IVa
clfA , hlg, pvl , tsst-1	IVc
hlg , pvl	IVa
hlg , pvl	IVc
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA, hlg, pvl	IVa
clfA,hlg, pvl	V
clfA, pvl	II

eta , hlg	NT
clfA, eta , hlg, tsst-1	NT
eta , hlg , tsst-1	NT
clfA, eta, hlg, pvl	IVa
eta , hlg, pvl	IVa
hlg, pvl, tsst-1	IVc
pvl	IVc
pvl	IVc
pvl	IVc
pvl	IVc
hlg	IVc
hlg	NT
hlg	NT
clfA	IVa
clfA	IVc

3.7 Genetic similarity analysis

The genetic similarity between *S.aureus* isolates , showed the isolates were classified into two main clusters (I-II) depending on the genetic relationship between *SCC*mec types and virulence factors genes, The main cluster I was the largest cluster, and it was divided into two subclusters, IA and IB. All isolates of subcluster IA were classified to the *SCC*mec IVc type. whereas the most isolates of subcluster IB were belonged to the *SCC*mecIVa type and were carried the gene encoding for Hlg. The cluster II was smallest cluster, The results observed that all isolates of cluster II belonged to *SCC*mec II type and did not carry the genes encoding for virulence factors except one isolate (B4) which carried genes encoding for Pvl, ClfA (table 5) (figure 4).

Table 5. Clustering and similarity of *S. aureus* isolates and number of isolates in each cluster

Clusters	Dendrogram Similarity in clusters	Number of isolates in each cluster (%)	Subclusters	Dendrogram Similarity in Subclusters	Number of isolates in each Subcluster (%)
I	23%	29(67.44%)	IA	32%	9(31.03%)
			IB	36%	20(68.96%)
II	33%	7(16.27%)			

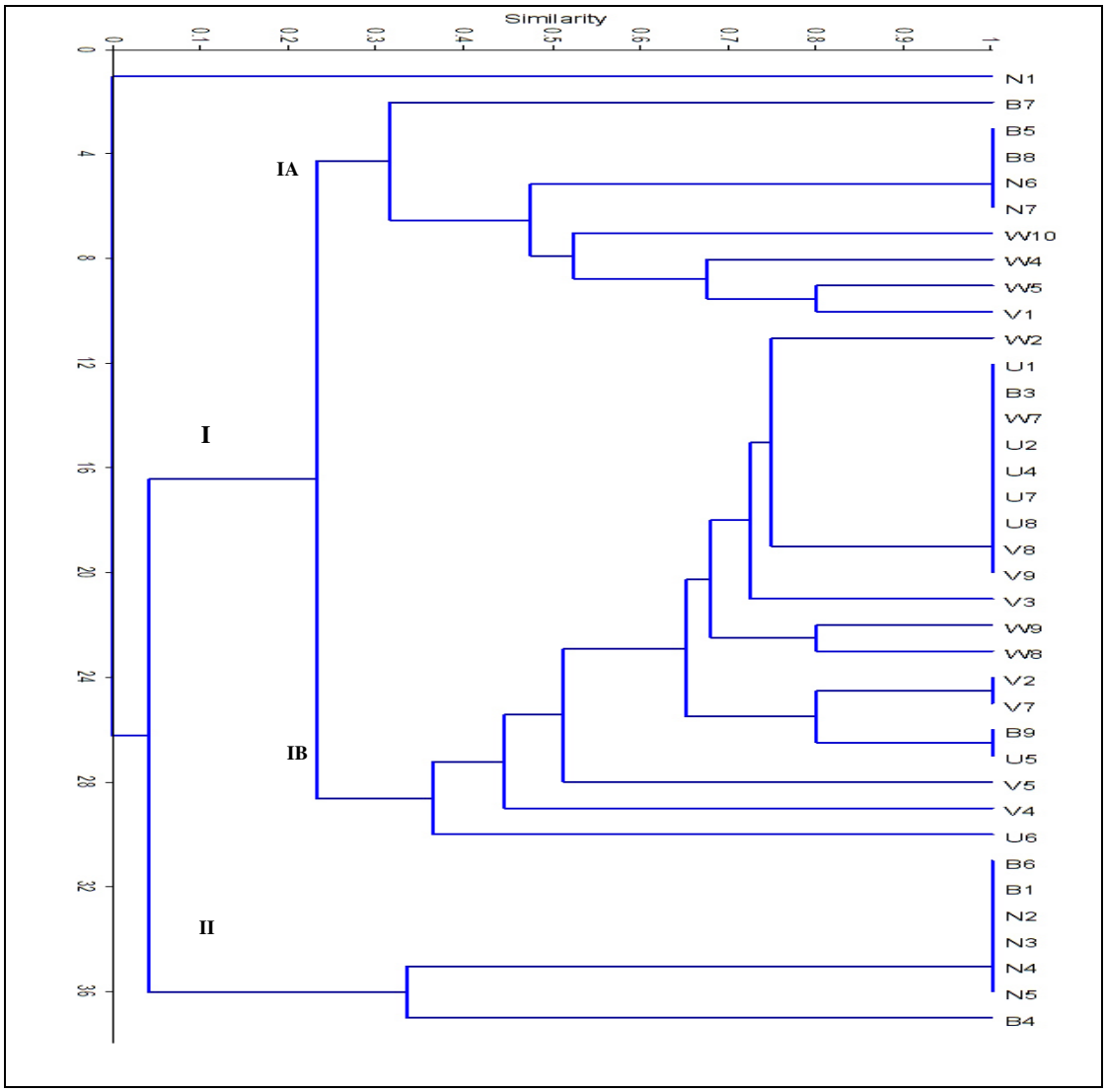

Figure 4. Dendrograms of genetic relationship among isolates of *S.aureus* were determined on the basis of the Jaccard similarity using Unweighted Pair-Group Method with Arithmetic Average (UPGMA)

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