World Journal of
Experimental Biosciences

Volume 5, Number 1: 23 - 29
ISSN: 2313-3937
2017

Research article

Use Multiplex PCR Technique for Distribution the Accessory Gene Regulatory Polymorphisms among Baghdad Clinical *Staphylococcus aureus* Isolates and its Correlation to Cassette Type

Hiba Abdulameer Mohammed[1]*, Nuha Joseph Kandala[1], Israa Al-Ogaidi[1]

ABSTRACT

Accessory gene regulator (agr) operon is the central transcriptional global regulatory system of Staphylococcus aureus; the agr locus is perceived as a part of the center genome of the S. aureus chromosome, responsible of controlling the virulence factors and cell-wall components. Considered the important quorums sensing system of S. aureus, agr activity is critical for skin and soft tissue infections; depending on its reported role in human and animals infections throw the up, down-controlling S. aureus infection advancement since up-control of virulence factors via agr is vital for disease advancement in some cases of intense infection, such infective endocarditis, skin and soft tissue infections for that there is global concern to develop drugs targeting this quorum sensing system. The objective of the study is typing the accessory genes regulator polymorphisms in Baghdad clinical isolates and its relation with the bacterial cassette types. Eighty seven isolates were collected from different sources and characterized by original biochemical tests and there susceptibility were tested toward several types of antimicrobial agents then they applied to molecular diagnosis to confirm characterization of S. aureus isolates by nuc gene with agr types, SCCmec types also several virulence factors and mec gene. It was found all isolates were community acquired harboring SCCmec type IV and one isolate have SCCmec type V also about 68.96% of the isolates were MRSA, most of isolates 75.86% were agr type I. Most MRSA isolates that isolated from Baghdad hospitals was CA-MRSA considering to they; Harboring SCCmec type IV and V., carrying accessory gene regulator (agr) type I., Have pvl gene, showed low susceptibility toward Vancomycin antibacterial agent, Also the studied isolates shown high similarity depending on their cassette type and accessory gene regulator polymorphisms. The most common virulence factors in local clinical isolates of S. aureus were clfA gene (61.6%) followed by pvl gene (32.55%), hlg gene (31.39%), icaA gene (27.58%), tsst-1 gene (24.41%) and eta gene (17.44%).

Keywords: S. aureus, agr, SCCmec, Quorum sensing system (QS), Virulence factors, pvl, tsst-1, MRSA, MDR.

Citation: **Mohammed HA, Kandala NJ, Al-Ogaidi I. (2017)** Use Multiplex PCR technique for Distribution the Accessory gene regulatory polymorphisms among Baghdad Clinical Staphylococcus aureus Isolates and its correlation to the Cassette Type. World J Exp Biosci **5**: 23 – 29.

Received November 25, 2016; Accepted January 13, 2017; Published February 14, 2017.

* Correspondence: : Mohammed HA.hibaabdulameer@gmail.com.
Department of Biotechnology, College of Science, University of Baghdad, aghdad, Iraq.

Full list of author information is available at the end of the article.

INTRODUCTION

Staphylococcus is a gram-positive cocci, non-spore forming, non motile, 0.5-1.5µm in diameter have genome size of 2.8Mbp with low (G+C) content, can form golden colonies by cause of producing carotenoids over growth, view in irregular clusters like grapes as a result of its three planes dividing capacity [1], capable of infect all tissues, Divide into two main types; methicillin sensitive *S.aureus* (MSSA) and methicillin resistance *S.aureus* (MRSA) [2] which developed from MSSA via receiving SCC*mec* complex containing *mec*A gene that responsible for extra penicillin binding protein that provided it with strong resistance to all ß-lactam antibiotics as long as other types in some cases [3]. MRSA isolates based on types of SCC*mec* can divide into two types; HA-MRSA and CA- MRSA strains. Types I, II, and III are most abundant with HA- MRSA, whereas types IV and V are found within CA-MRSA strains [4]. Accessory gene regulator (*agr*) operon is the central transcriptional global regulatory system of *S.aureus;* the *agr* locus is perceived as part of the center genome of *S.aureus* chromosome not a pathogencity island. With 3.5 kb content and comprises of two unique separate transcriptional units, RNAII and RNAIII, that translation is forced by the P2 and P3 promoters, individually [5]; responsible of controlling the virulence factors and cell-wall components by action of RNAII and RNAIII. It considered as one of the quorums sensing system of *S.aureus* .Many reports suggested that *agr* activity is critical for skin and soft tissue infections; depending on its reported role in human and animals infections for that there is global concern to develop drugs targeting this quorum sensing system [6]. As [7,8] reported the (up, down)-controlling of *agr* system is effecter in *S.aureus* infection advancement since up-control of virulence factors via *agr* is vital for disease advancement in some cases of intense infection, such infective endocarditis, skin and soft tissue infections [9,10], pneumonia [11], and septic arthritis and osteomyelitis. The *agr* operon Sequencing is not similar in all *S.aureus* strains, four general variants, designated *agr* I to IV [12]. Furthermore, the *agr* type I–IV polymorphisms were assessed as a variable that inclines the lastingness and survival of MDR *S.aureus* clinical isolates amid nosocomial or hospital-acquired infections [13]. The *agr* controlling expression of virulence factors is energy-consuming mechanism and recently some reports demonstrating that this should be adjusted with the expression of antibiotic resistance in antibiotic- resistant strains specially using of antibiotics sub-inhibitory concentration results enhancing *agr* activity [14,15]. The aim of present study is evaluating the molecular diagnosis of clinical isolates, *agr* polymorphisms and its relation with the bacterial cassette types.

MATERIALS and METHODS

Collection of clinical Specimens

Eighty seven clinical samples were obtained from different clinical specimens such as urine, blood, wound and nose that collected from local different hospitals obtained from different age's male and female patients with Urinary Tract Infection, Bacterimia, Wound Infection, Burns, and Nasal Infection. All isolates were identified by standard microbiological techniques then characterized by molecular analysis. Collected swabs were streaked on BHIA, mannitol agar, blood agar; they were incubated at 37C° for 24 h; the suspected colonies depending on the morphological bases were selected for further diagnostic tests.

Antimicrobial susceptibility test

Susceptibility of *S. aureus* isolates toward several antimicrobials was determined by Kirby-Bauer disk diffusion method to survey the evaloated resistance of the *S. aureus* isolates to more antimicrobial agents that used as traditional therapy.

Genomic DNA extractions and measuring

Genomic DNA of the bacterial isolates was extracted using Exiprep Genomic DNA kit supplied by (Bioneer/Korea),The concentration and purity measured using nanodrop provide by ACT Gene\Korea. The DNA samples integrity was tasted using gel electrophoresis method.

Molecular analysis of genomic DNA using multiplex PCR technique

The molecular diagnosis was done using Thermo Cycler Machine (Multigene\USA) with primers supplied by (Promega\USA, KAPA\USA). The reaction mixture was set up as follows: (1X) of GoTaq®Green Master Mix (promega/USA) which contain Taq DNA polymerase, $MgCl_2$, (dNTP), reaction buffer with two dyes, different concentration of each used primer (10 pmol) (**Table 1**), 100 ng of DNA template and sterile distilled water was added to reach the final volume, under cold aseptic conditions in laminar air flow cabinet (Techne\UK). After the amplification (10 µl) of PCR amplified products were separated by electrophoresis in 2.5 %(w/v) Agarose gel in 1X TBE buffer. Gel was run at 90 V for 60 min after staining with Ethidium bromide they visualized under UV illumination, and imaged by gel documentation apparatus (ATTO\Korea). The products size was estimated by comparing with marker 100 bp DNA ladder provided by (KAPA\USA).

RESULTS

Susceptibility Test

Results of antimicrobial susceptibility profiles showed that study isolates were multidrug resistance (MDR) that resist at least seven antibiotics using disk diffusion method susceptib- ility test of eighty seven isolates were done toward 26 antimicrobial drugs of different groups; ß-lactam group (Ampicillin, PencilinG, Oxacillin, Methicillin, Ampicillin\ Cloxacillin, Cefaclor, Cefoxitin, Ceftriaxone, Cephalothin, Bacitracin) which showed resistance in percentage 100, 100, 81,74, 100, 100, 100, 6, 53, 17%, respectively, Amino- glycosides group, Amikacin, Gentamicin, Streptomycin, Tobramycin, Kanamycin, Neomycin were 92,92,61,53, 63, 84
%, respectively. Macrolides group, Erythromycin, Azythromycin and Clarithromycin were 70, 75 and 66%, respectively. Quinolones group, Ciprofloxacin and Norfloxacin were 9 and 46 %, respectively. Glycopeptides group, Vancomycin was 14%. Whereas toward Carbapenem group, Imipenem was 100
% susceptible. That agrees with the local study of Abdul-Wahhab (2014) that mention Baghdad *S. aureus* were MDR.

Molecular Diagnosis of S.aureus Isolates Using Multiplex PCR Technique

Molecular Diagnosis of nuc and mecA Genes

Results of the duplex PCR reaction showed that MRSA (60 isolates) appeared with two bands one with molecular weight

Table 1. Primers used in the current study and their sequences

primer	Sequence 5'……3'	Amplicon size	Reference
nuc-F	GCGATTGATGGTGATACGGTT	276 bp	
nuc-R	AGCCAAGCCTTGACGAACTAAAGC		
*mec*A-F	GTGAAGATATACCAAGTGATT	147 bp	
*mec*A-R	ATGCGCTATAGATTGAAAGGAT		Zhang *et al*.,(2005)
Type I-F	GCTTTAAAGAGTGTCGTTACAGG	613 bp	
Type I-R	GTTCTCTCATAGTATGACGTCC		
Type II-F	CCATATTGTGTACGATGCG	398 bp	
Type II-R	CGAAATCAATGGTTAATGGACC		
Type III-F	CCATATTGTGTACGATGCG	280 bp	
Type III-R	CCTTAGTTGTCGTAACAGATCG		
Type IVa-F	GCCTTATTCGAAGAAACCG	776 bp	
Type IVa-R	CTACTCTTCTGAAAAGCGTCG		
Type IVb-F	TCTGGAATTACTTCAGCTGC	493 bp	
Type IVb-R	AAACAATATTGCTCTCCCTC		
Type IVc-F	ACAATATTTGTATTATCGGAGAGC	200 bp	
Type IVc-R	TTGGTATGAGGTATTGCTGG		
Type IVd-F	CTCAAAATACGGACCCCAATACA	881 bp	
Type IVd-R	TGCTCCAGTAATTGCTAAAG		
Type V-F	GAACATTGTTACTTAAATGAGCG	325 bp	
Type V-R	TGAAAGTTGTACCCTTGACACC		
Pan agr	ATGCACATGGTGCACATGC	441 bp	
agr I	GTCACAAGTACTATAAGCTGCGAT		Gilot *et al*., (2002)
agr II	TATTACTAATTGAAAAGTGGCCATAGC	575 bp	
agr III	GTAATGTAATAGCTTGTAAAAAGTGGCCATAGC	323 bp	
agr IV	CGATAATGCCGTAATACCCG	659 bp	
clfA-F	ATTGGCGTGGCTTCAGTGCT	292 bp	Tristan *et al*., (2003)
clfA-R	CGTTTCTTCCGTAGTTGCATTTG		
eta-F	ACTGTAGGAGCTAGTGCATTTGT	190 bp	Jarraud *et al*., (2002)
eta-R	TGGATACTTTTGTCTATCTTTTTCATCAAC		
hlg-F	GTCATAGAGTCCATAATGCATTTAA	535 bp	
hlg-R	CACCAAATGTATAGCCTAAAGTG		
pvl-F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433 bp	
pvl-R	GCATCAAGTGTATTGGATAGCAAAAGC		
tsst-1-F	GCTTGCGACAACTGCTACAG	559 bp	Monday and Bohch,(1999)
tsst-1-R	TGGATCCGTCATTCATTGTTAT		
icaA-F	AAACTTGGTGCGGTTACAGG	750bp	Szczuka *et al*., (2012)
icaA-R	TCTGGGCTTGACCATGTTG		

of 267 bp of positive amplified for specific primer of *nuc* gene and a second band of 147bp for *mec*A gene specific primer. In the time that MSSA (27 isolates) appear with solo band of molecular weight about 267 bp of *nuc* gene specific primer amplifying with absent of *mecA* gene.

Molecular Diagnosis of Cassette Typing and Sub-typing of S. aureus Isolates by Detection of SCCmec Genes

Multiplex PCR reaction was done for detected the *SCCmec* types of the 60 studied MRSA isolates (68.96%) with two separate reactions; the founds showed absence of types (I, II, III, IVb, IVd) among the study isolates which are divided into; one (1.66%) was type V and 10 isolates (16.66%) was subtype IVc and the 49 isolates (81.66%) was subtype as IVa (**Fig 1**).

Molecular Diagnosis of Accessory Genes Regulatory system Typing of S.aureus Isolates

Typing the accessory gene contain of the studied isolates done via detection the *agr* type in du-PCR reactions and the results were 66, 3, 1 and 3 of *agr* types I, II, III and IV, respectively; and fourteen isolates (16.09%) showed no existence of any *agr* polymorphisms (**Fig 2**).

As a result of the expected amplicon size; founds shown existence of *agr* distant polymorphisms as follows: among 60 MRSA isolates 45 isolates was belong to *agr* type I, 3 isolates was *agr* type II, none of isolates was *agr* type III, 2 isolates was *agr* type IV and 10 isolates showed no existence of *agr* genes. While the MSSA isolates showed that 21 isolates among the 27 isolates were belong to *agr* type I, none of the isolates was *agr* type II, 1 isolate of each *agr* type III and *agr* type IV finally 4 isolates was absence of *agr* genes. The distribution of accessory genes polymorphisms with the SCC*mec* types among the 87 *S. aureus* isolates was as the following: 36 isolates have SCC*mec* type IVa were positive for *agr* I, eight isolates was SCC*mec* type IVc and *agr* I finally one isolate of SCC*mec* type III was *agr* I. While, *agr* II polymorphism was appeared in 3 isolates that shown SCC*mec* type IVa, none *agr* II detect in each SCC*mec* types (IVc and III). *agr* type IV was detect in two isolate; one in SCC*mec* type IVa and one in SCC*mec* type IVc. While, the *agr* type III polymorphism was absent in all MRSA isolates (**Fig. 3**).

Molecular Diagnosis of Some Selected Virulence Factors of *S. aureus* Isolates

Molecular diagnosing of the *S. aureus* isolates under study shown that the most virulence factor existing is *clfA* gene in 53 isolates followed by *pvl* gene in 28 isolates, *hlg* gene found in 27 isolates, *ica*A gene in 24 isolates, *tsst-1* gene in 21 isolates and *eta* gene in 15 isolates. Finally, only 6 isolates showed absence of all selected virulence factors (**Fig. 4**).

Cluster analysis

A dendograme was constructed based on dice coefficient genetic similarity of information by using UPGMA cluster analysis (Rohlf, 1998) showing the similarity of community acquired *S.aureus* isolates based on the genetic relationship among *agr*, SCC*mec* and virulence factors (**Fig 5**).

DISCUSSION

The results hinted that all isolates of *S. aureus* (87 isolates) that previously characterized with conventional biochemical tests were successfully amplified by the *nuc* gene but not the *S. epidermidis* that used as negative control which mention the

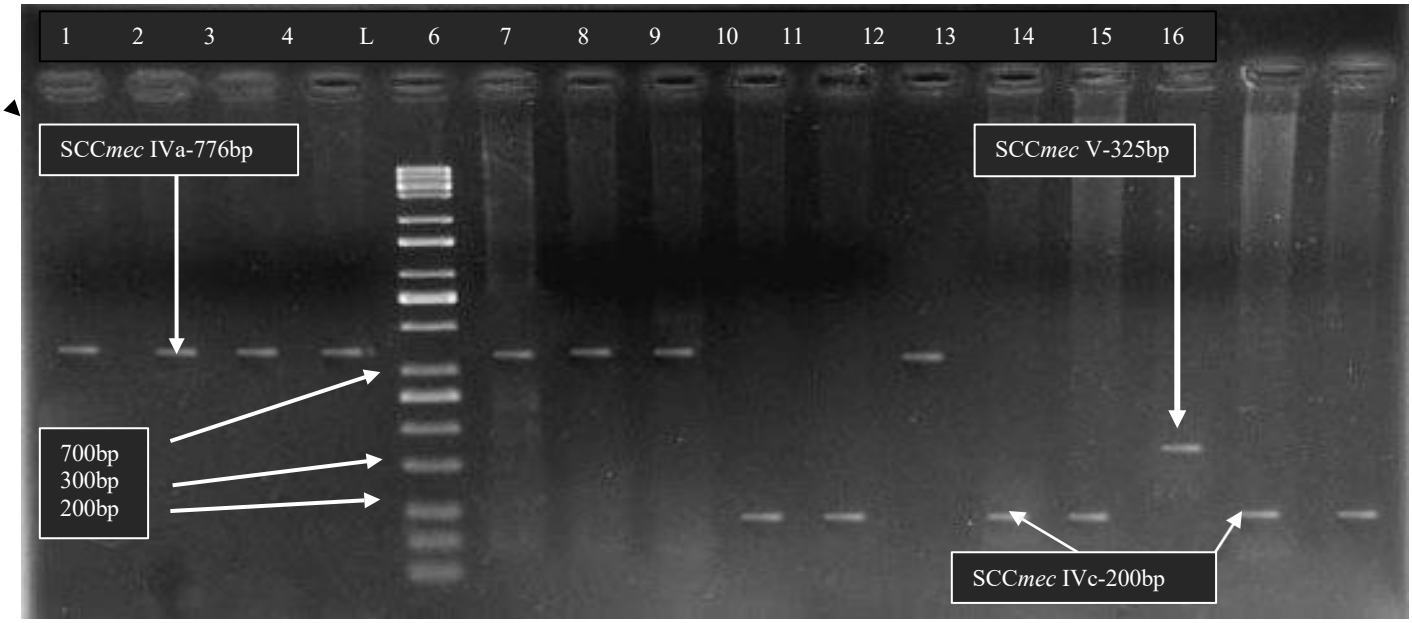

Fig 1. Agarose gel electrophoresis of PCR amplification products of *S.aureus* isolates SCC*mec* genes;Using Ladder of molecular size (100-10000bp) line(5),lines (1,2,3,4,6,7,8,11) represent SCC*mec* type IVa, with band (776bp) and lines (9,10,12,13,15,16) present isolates with SCC*mec* type IVc with band (200bp) and line (14) represent SCC*mec* type V with band (325bp);separated on 2% Agarose gel (80V, 1X TBE buffer) for 1h.

specify of *nuc* gene for *S. aureus* among other *staphylococcus spp*. [17,18]. From 60 MRSA 44 isolates showed methicillin resistance in susceptibility test and 16 isolates showed methicillin sensitivity. That due to for sometimes, *mec*A gene was in-vivo expression gene; in addition, the expression of *mec*A is lower in planktonic bacterial cells than the others [19]; or it as a result of their inefficiency to deliver enough PBP2a [20]. At the same time, the 27 MSSA isolates that not carry *mec*A gene; ten of them showed methicillin resistance in susceptibility test which referred they have another alternative mechanisms instead of *mec*A gene such as: additional genetic components can be available like, plasmids and some acquired chromosomal resistance genes toward ß-lactam agents and heavy metals [21], overdone generation of ß-lactamase in these isolates or development of normal PBP2 proteins with changed capacity for connection or production of alternative PBP2a protein [22]. plus, the resistance of the isolates by cause of the exit of *blaZ* gene expression and trans-membrane proteins that expressed by *Mec*R1 and *Bla*R1 genes [23]. In the other hand, there are a few environmental conditions can in like manner effect the methicillin resistance,

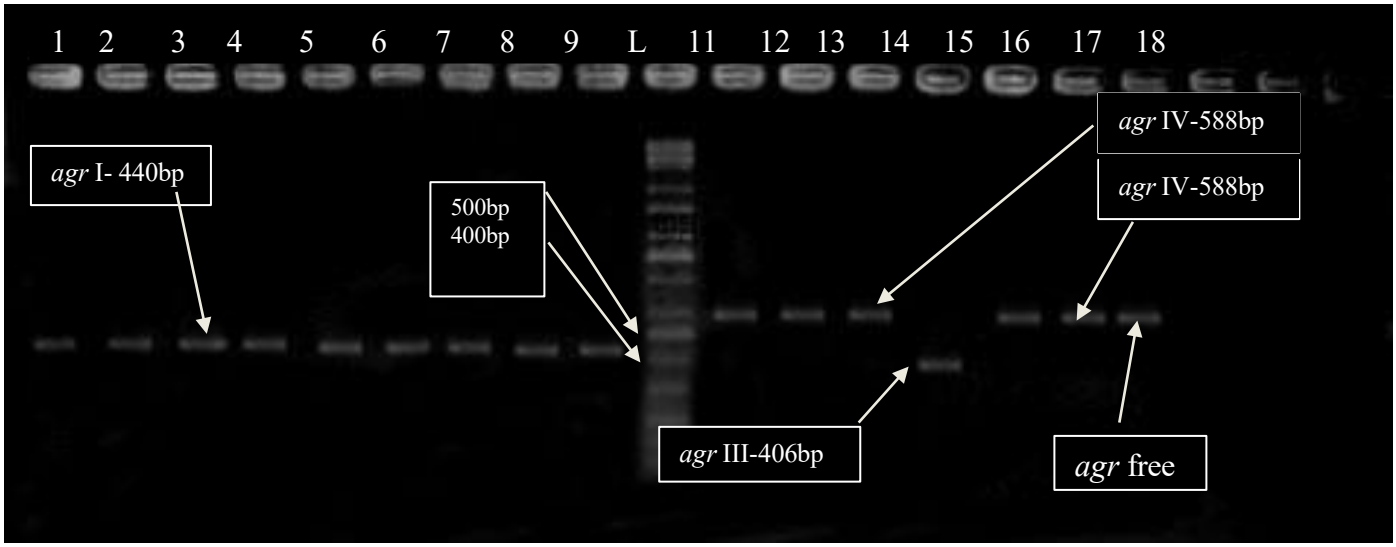

Fig 2. Agarose gel electrophoresis of PCR amplification products of *S.aureus* isolates *agr* accessory genes (440bp *agr* I gene, 572bp *agr* II gene, 406bp *agr* III gene, 588bp *agr* IV gene); Using Ladder of molecular size (100-10000bp) line (10), lines (1- 9) represent *agr* I and lines (11,12,13) present isolates with *agr* IV polymorphism, line (14) present *agr* III, lines (15,16,17) present *agr* II and lines (18, 19, 20) present isolates free of *agr* genes; separated on 2.5% Agarose gel (90V, 1X TBE buffer) for 1h visualized using U.V. light after staining with ethidium bromide.

as the temperature, pH and level of NaCl contain in media [24], for all reason above, it cannot depend only on phenotype screening to detect MRSA isolates.

Current study reported that *agr* I is the most frequent accessory gene polymorphisms in both MRSA and MSSA isolates which is similar to previous study [25], it was showed that the *agr* I is the most frequent polymorphism in Iranian isolates flowed by *agr* II and closely to previous study [26] that showed from 106 Egyptian isolates (74.4%) of the isolates was *agr* I, (15.4%) *agr* II, (10.2%) *agr* III and none of *agr* IV also this finding is closely to results of previous study [27], which reported that most of the Belgium isolates (72.1%) were *agr* I.

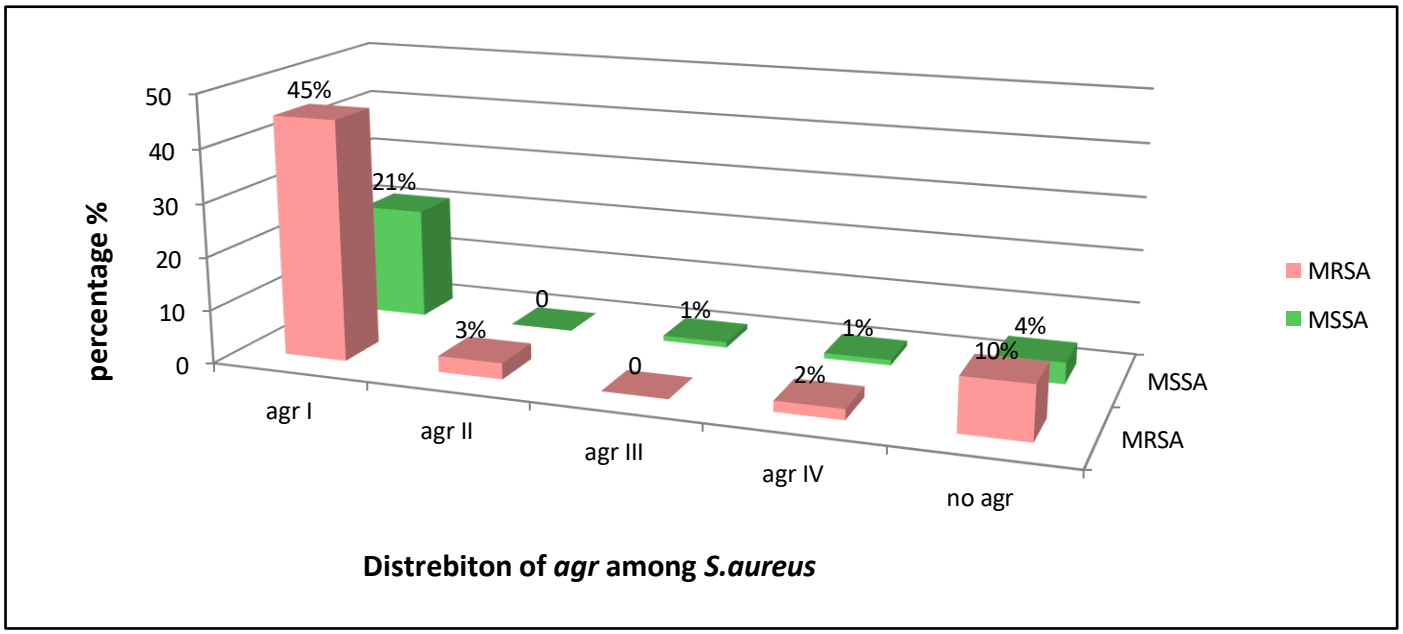

Fig 3. Relationship between accessory genes and Cassette in *S. aureus.*

Multiplex PCR showed that the most present virulence factors in Baghdad *S. aureus* clinical isolates was *clfA* gene (61.6%) followed by *pvl* gene (32.55%), *hlg* gene (31.39%), *ica*A gene (27.58%), *tsst-1* gene (24.41%) and *eta* gene (17.44%). This foundation is in agreement with reports from different places over the world that showed the lack of classical risk factors in MRSA isolates from patients have SCC*mec* IV [28]. Still, the clinical CA-MRSA isolates showed more resistance activity than the screening *SCC*mec IV as reported previously [29]. Summation of the study results suggests that all 87 *S. aureus* isolates were CA-MRSA. Since *pvl* gene existence in genotype of MRSA considered as marker for CA-MRSA infections, that Presence in around 33% of the isolates support the idea that the studied isolates are CA-MRSA [30] proved; despite the fact that there are reports on CA-MRSA without *pvl*. In addition to the fact that SCC*mec* IV is a marker for CA-MRSA [31,12],

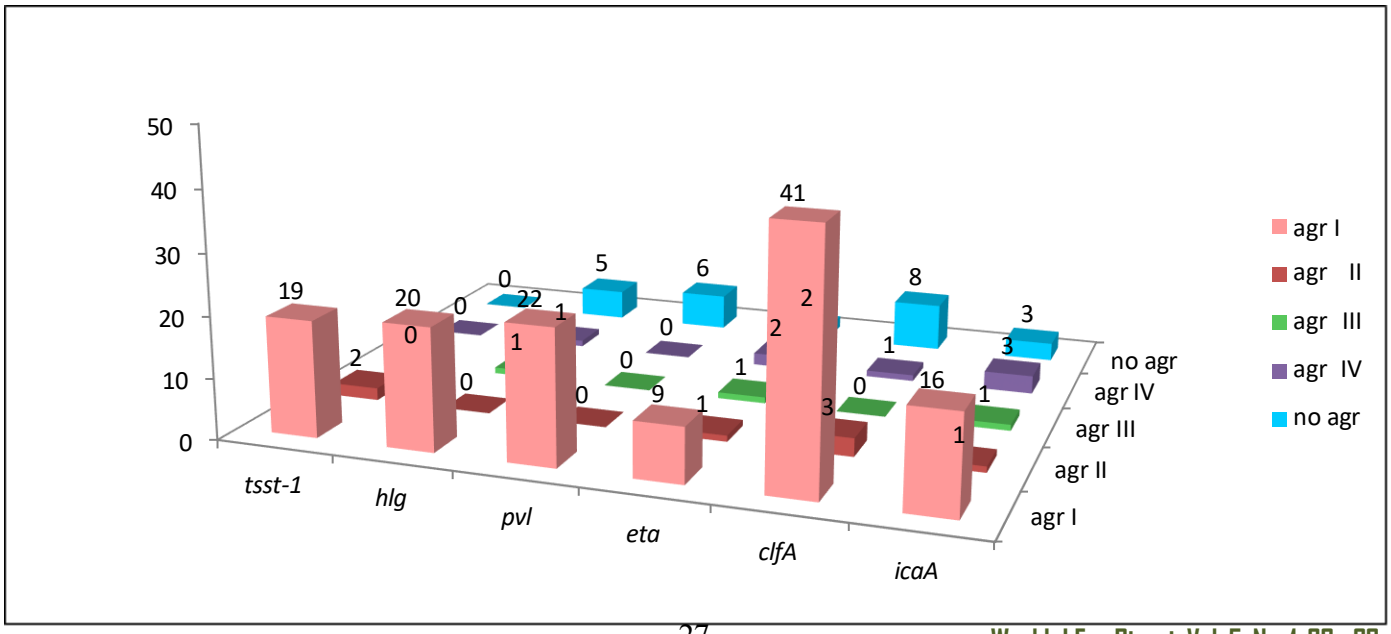

Fig 4. Relationship between accessory genes polymorphism and virulence factors.

which reported that most CA-MRSA isolates over world are contain *agr* I plus the most of the isolates showed high susceptibility toward Vancomysin antibiotic, so, all these reasons enhance that the Baghdad studied *S.aureus* isolates are CA-MRSA. Most of Baghdad methicillin resistant *S. aureus* isolates was CA-MRSA considering to they; Harboring SCC*mec* type IV and V., Carrying accessory gene regulator (*agr*) type I., Have *pvl* gene, showed low susceptibility toward Vancomycin antibacterial agent. The studied isolates showed high similarity depending on their cassette type and accessory gene regulator polymorphisms. Most common virulence factors in Baghdad *S. aureus* clinical isolates was in fact *clfA* gene (61.6%) followed by *pvl* gene (32.55%), *hlg* gene (31.39%), *ica*A gene (27.58%), *tsst-1* gene (24.41%) and *eta* gene (17.44%).

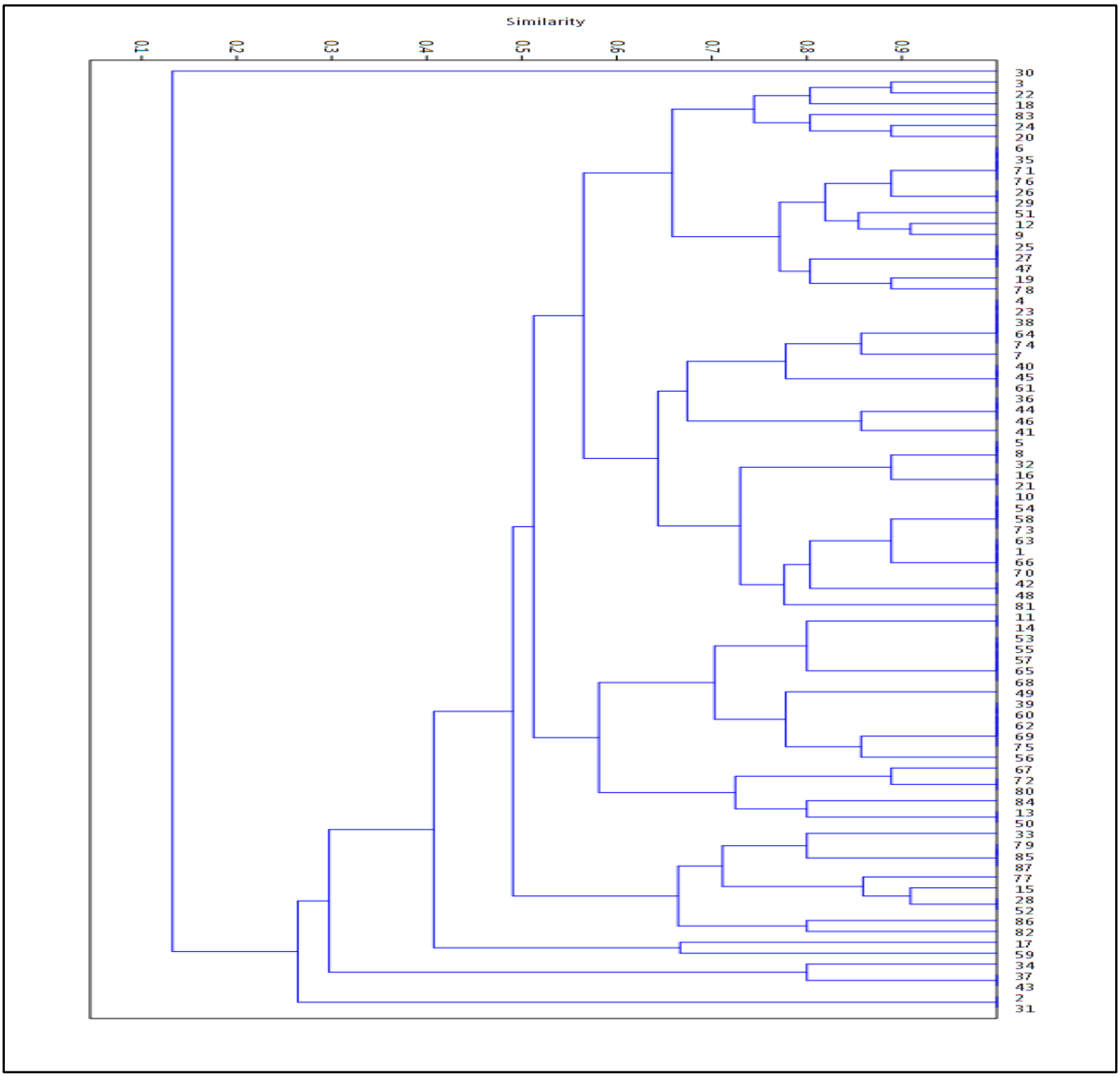

Fig 5. Dendrogram of genetic relationships among *S.aureus* isolates based on Dice coefficient genetic similarity.

Acknowledgment

The author would like to thank Wedean AL-Hadban for her nice support and help and Muneam R. Ali for his providing the cluster analysis program.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCES

1. Torres-Sangiao E, Holban AM, Gestal MC. (2016) Advanced Nanobiomaterials: Vaccines, Diagnosis and Treatment of Infectious Diseases. *Molecules* 21:867.
2. Bonar E, Wojcik I, Jankowska U, Kedracka-Krok S, Bukowski M, et al. (2016) Identification of Secreted Exoproteome Fingerprints of Highly-

Virulent and Non-Virulent *Staphylococcus aureus* Strains. *Front Cell Infect Microbiol* 6: 1-15.
3. Palavecino EL. (2013) Clinical, Epidemiologic, and Laboratory Aspects of Methicillin-Resistant *Staphylococcus aureus* Infections. *Methods Mol Biol* 1085:1-24.
4. Chambers HF, Deleo FR. (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Rev Microbiol* 7: 629-641.
5. Le KY, Otto M. (2015) Quorum-sensing regulation in staphylococci-an overview. *Front Microbiol* 6:1174.
6. Kobayashi SD, Malachowa N, Whitney AR, Braughton KR, Gardner DJ, et al. (2011). Comparative analysis of USA300 virulence determinants in a rabbit model of skin and soft tissue infection. *J Infect Dis* 204:937-941.
7. Periasamy S, Chatterjee SS, Cheung GY, Otto M. (2012) Phenol- soluble modulins in *staphylococci*: what are they originally for?. *Communicative Integrat Biol* 5:275–277.
8. Wang B, Muir TW. (2016) Regulation of Virulence in *Staphylococcus aureus*: Molecular Mechanisms and Remaining Puzzles. *Cell Chem Biol* 23:214-224.
9. Cheung GY, Wang R, Khan BA, Sturdevant DE, Otto M. (2011) Role of the accessory gene regulator *agr* in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. *Infect Immun* 79: 1927-1935.
10. Wright JSIII, Jin R, Novick RP. (2005) Transient interference with staphylococcal quorum sensing blocks abscess formation. *PNAS* USA 102:1691-1696.
11. Montgomery CP. (2012) CodY deletion enhances in vivo virulence of community-associated methicillin-resistant *Staphylococcus aureus* clone USA300. *Infect immune* 80:2382-2389.
12. Gray MG, Wholy WY, Wagner NO, Cremers SM, Mueller-Schickert A. (2014). Poly phosphate is a primordial chaperone. *Molecular cell*. 53:689-699.
13. Guillet J, Hallier M, Felden B. (2013). Emerging Functions for the *Staphylococcus aureus* RNome. *PLOS Pathogens*. 9.
14. Vicente MC, Prim DJ, Eberle AB, Visa N. (2015). RRP6/EXOSC10 is required for the repair of DNA double-strand breaks by homologous recombination. *Journal of Cell Science*. 128: 1097-1107.
15. Rudkin JK, Laabei M, Edwards AM, Joo HS, Otto M, Lennon KL, OGara JP, Waterfield NR, Massey RC. (2014). Oxacillin alters the toxin expression profile of community-associated methicillin- resistant *Staphylococcus aureus*. *Antimicrobial Agents Chemotherapy*. 58:1100-1107.
16. Joo HS, Otto M. (2012). Molecular basis of in-vivo biofilm formation by bacterial pathogens. *Chemistry and Biology*. 19: 1503–1513.
17. Rajeh A, Al-Achkar K, Al-Marin A, Safi M. (2015). Role of Polymerase Chain Reaction (PCR) in the detection of antibiotic- resistant *Staphylococcus aureus. Egyptian Journal of Medical Human Genetics*. 15:293-298.
18. Eliezer D, Townnsend SS, Sawyer PJ, Major B, Mendes WB. (2011). System justifying beliefs moderate the relationship between perceived discrimination and resting blood pressure. *Social Cognition*. 29:303-321.
19. Vandecasteele B, Lauriks B, De Vos B, Tack FMG. (2003). Cd and Zn Concentration in Hybrid Poplar Foliage and Leaf Beetles grown on polluted Sediment-derived soils. *Environment Monit. Assess*. 89:263-283.
20. Murakami S, Seki T, Wakabayashi K, Arai Y.(1991). The Ontogeny of Luteinizing Hormone-Releasing hormone (LHRH) Producing neurons in the chick embryo possible evidence for migration LHRH neurons form the Olfactory epithelium expressing a highly poly sialylated neural cell adhesion molecule. *Neuroscience*. 12:421-431.
21. Souza SG, Campos GB, Oliveira PS, Sousa DS, Silva DCCD, Santos VM, Amorim1 AT, Santos AMOG, Timenetsky J, Cruz1 MP, Yatsuda R, Marques LM. (2014). Virulence Factors in Methicillin-Resistant *Staphylococcus aureus* isolated from ICU Units in Brazilian. *Advance in Microbiology*. 4:207-215.
22. Wallet F, Roussel-Devallez M, Courcol RJ. (1996). Choice of a routine method for detecting methicillin-resistance in *staphylococci*. *Journal of Antimicrobial Chemotherapy*. 37:901-909.
23. Manjunath S, Padma T. (2012). Methicillin Resistant Staphylococcus aureus: Resistance Genes and Their Regulation. *International Journal of Pharmacy and Pharmaceutical Sciences*. 4:658-667.
24. Sabath LD. (1987). Some historical aspects of bacterial resistance. *Journal of Urban Health*. 63:330-336.
25. Ghasemian A, Shahin Najar Peerayeh SN, Bakhshi B, Mirzaee M. (2015). Detection of accessory gene regulator groups genes and cassette chromosome *mec* types among *Staphylococcus aureus* isolated from intensive care unit patients. *Asian Pacific Journal Tropical Disease*. 5:153-157.
26. Melake NA, Zakaria AS, Ibrahim NH, Salama MA, Mahmoud AZ. (2014). Prevalence of *agr* specificity groups among in vitro biofilm forming methicillin resistant *Staphylococcus aureus* strains isolated from nasal carriers. *International Journal of Microbiology Research*. 5:76-84.
27. Bardiau M, Detilleux J, Farnir FR, Mainil GJ, Ote I. (2014). Associations between properties linked with persistence in a collection of *Staphylococcus aureus* isolates from bovine mastitis. *Veterinary of Microbiology*. 169:74-79.
28. Wang JL, Wang JT, Chen SY, Chen YC, Chang SC. (2010). Distribution of Staphylococcal cassette chromosome *mec* types and correlation with co-morbidity and infection type in patients with MRSA bacteremia. *PLOS ONE*. 5(3): e9489.
29. Shitrit N, Yulevich I, Maguid E, Ozeri D, Veksler D, Kleiner V, Hasman E. (2013). Spin-optical metamaterial route to spin-controlled photonics. *Science journal*. 340:724-726.
30. Abimanyu N, Krishnan A, Murugesan S, Subramanian SG, Gurumurthy S, Krishnan P. (2013). Use of Triplex PCR for Rapid Detection of PVL and Differentiation of MRSA from Methicillin Resistant Coagulase Negative Staphylococci. *Journal of Clinical and Diagnostic Research*. 7:215-218.
31. Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. (2012). Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). *Current Opinion in Microbiology*. 15:588-595.

Author affiliation
1. *Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.*


Unicorn