Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (1):387-393
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Distribution of enterotoxin genes and AGR types in *Staphylococcus aureus* (Enterotoxins and AGR types in *S.aureus*)

Alka Hasani[1], Milad Sabaee[1*], Mohammad Ahangarzadeh Rezaee[2], Akbar Hasani[3], Siroos Jedari Seifi[2] and Nasim Asadi Faezi[1]

[1]*Infectious and Tropical Diseases Research Center, and Department of Medical Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Department of Medical Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Drug Applied Research Center and Department of Clinical Biochemistry and Laboratory Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

ABSTRACT

Staphylococcus aureus is a major human pathogen harboring several virulence factors and super antigens (Sag) or staphylococcal enterotoxins (SEs). These factors are associated with the presence of agr (Accessory Gene Regulator) locus that controls the expression of several of them. We aimed to determine the prevalence of super antigens and agr groups in 151 methicillin resistant and sensitive S. aureus and to investigate possible relationships between them. A series of six multiplex PCR were conducted. Gradual increase in methicillin resistant S. aureus isolates was evident in this study. Additionally, steady decline in agr II, III and IV types with emergence of agr type I as predominant type was a significant feature. Seventy nine toxin genotypes were observed, with the most common genotype being sea-sek-seq. A meaningful relationship between the major toxin genes, methicillin resistance and agr type 1 was observed. Enterotoxin gene cluster (egc)1 was more common than egc2. The overall prevalence of virulence factors corresponds with that seen in other European countries; however preferential association in this study between agrI and sea-sek-seq combination may serve as efficient triggering signal of virulence in clinical S.aureus isolates.

Key words: *Staphylococcus aureus*; Accessory gene regulator; Enterotoxin gene cluster; Methicillin resistance

---

INTRODUCTION

Staphylococcus aureus exploits diverse pathogenic factors to damage tissue, facilitates colonization and produce systemic diseases [3] and in return helps itself to tolerate the difficult conditions inside its host. In healthy individuals, *S. aureus* ingeniously retains the regulation of these virulence determinants and withholds itself from causing severe infection [5]. The last decade focused on the role of staphylococcal exoproteins, such as super antigens and cytolysins, which are of special interest in the establishment and spreading of infection [18, 24]. Though traditional therapeutic antibiotics do not influence the production of exoproteins or neutralize them in host, but have been hypothesized to aggravate the patient's condition by inducing the production of other harmful exoproteins and cytolysins if used inadequately, particularly for methicillin resistant *S.aureus* (MRSA) infections in comparison to methicillin sensitive *S.aureus* (MSSA) [22].

Almost all *S. aureus* strains produce and release a number of cytotoxins and enzymes, mainly to facilitate the bacterial growth in host tissue, by turning it into suitable and nutritious medium. Some strains also produce toxins with super antigenic activities, which include staphylococcal enterotoxins (SEs) (SEA, SEB, SECn, SED, SEE, SEG, SEH, and SEI), the exfoliative toxins (ETA and ETB) and toxic shock syndrome toxin-1 (TSST-1).

Molecular basis of pathogenicity of this organism includes expression of a cluster of accessory genes located on *agr* locus (the locus regulates the expression of most virulence factors in *S. aureus*), cell wall-associated proteins and extracellular proteins [23]. *agr* locus encodes a signaling pathway with two components which is in turn activated by a quorum-sensing signaling peptide (autoinducing peptide) [29]. Based on the specificity of the autoinducing peptide to its membrane receptor, *S. aureus* strains are categorized into four *agr* groups (I-IV) [25]. While isolates of one specific *agr* group are able to activate the *agr* locus of other isolates of the same *agr* group, they have inhibitory effects on *agr* response in isolates of other *agr* groups, so called a novel form of bacterial interference [10]. The resulting influence of *agr* autoiducer-receptor specificity on enhancement and inhibition of *S. aureus* colonization in host is being suggested a special kind of relationship between *agr* polymorphism, epidemiology and transmission of *S. aureus* clones [31].

Systematic review performed in Iran shows moderate heterogeneity in relative frequency of MRSA [2]. However, there exists a paucity of literature on genotypic characteristics and other attributes of the *S. aureus* isolates. Though an understanding exist on the presence of exotoxins of *S.aureus* isolates obtained from nasal cultures elsewhere [13], however, a lacuna exists in the type of exotoxins possessed by clinical *S.aureus* isolates in Iran. Our hospital serves patients from Northwest Iran and each year *S.aureus* strains are being stored for further evaluation. Thus, we aimed to characterize *S. aureus* isolates obtained from University teaching hospital in Tabriz, Iran between the years 2007 and 2014 with regard to (i) the comparative prevalence of enterotoxin (SEs) genes in MRSA and MSSA, ii) prevalence of *agr* types, and iii) to determine possible relationships between the presence of SEg genes and different *agr* groups in these isolates.

MATERIALS AND METHODS

Bacterial strains and confirmation of speciation: The work was conducted on 151 randomly selected *S.aureus* isolates obtained from various clinical infections in patients admitted to the University Teaching Hospital, between years 2007-2014, and stored at -70° C in 20% glycerol broth for future use. After thawing the bacterial sample, the identities of all strains were reconfirmed by conventional biochemical methods and at molecular level by using *nuc* gene as described previously [17].

Antibiotic susceptibility test and confirmation of methicillin resistance: The antibiotic susceptibility was performed as a qualitative test as described earlier [17] using Kirby-Bauer (disc agar diffusion) method. The panel of antibiotic disks used was as follows: penicillin (P) (10unit), gentamicin (Gm) (10µg), trimethoprim-sulphamethaxazole (TMP-SXT) (25µg), rifampin (RM) (5µg) , linezolid (LZ) (30µg), ceftriaxone (CRO) (30µg) ciprofloxacin (CP) (5µg), ofloxacin (OF) (5µg), imipenem (IMI) (10µg), meropenem (MRP) (10µg), teicoplanin (TE) (30µg), azithromycin (AZM) (15µg), erythromycin (ER) (15 µg) and clindamycin (CD) (2 µg) and results were interpreted according to CLSI (Clinical and Laboratory Standards Institute) [39] criteria.

Detection of methicillin-resistance in *S.aureus* strains was carried out by disc agar diffusion test using 30 µg cefoxitin disk, and detecting the presence of *mec*A gene as described previously [17, 32]. Vancomycin resistance was evaluated as Minimum Inhibitory Concentration (MIC) assay as per CLSI recommendation using E-test strips [39]. Multi Drug Resistance (MDR) was defined as concomitant resistance to at least three different antibiotic classes.

Amplification of SEg and *agr* locus genes by Polymerase chain reaction- (PCR)
DNA extraction was carried out using CinnaGen DNG kit (Cinnagen, Iran) with a slight modification. Lauria -broth medium inoculated with *S.aureus* and incubated for 24 hours was centrifuged and the pellet suspended in Tris – EDTA buffer for10 minutes at 95°C. The procedure was followed by the instructions provided with the above mentioned kit. Finally the supernatant containing the extracted DNA was stored at -20°C for the molecular tests.

A set of six multiplex PCR was performed on each isolate for the SE genes including; *sea, sec, seh, sed, sek, see, seb, sem, sel, seo, sen, seg, seq, sej, sei, ser, seu* and *sep*, and *agr* types; *agr-1, agr-2, agr-3* and *agr-4* using the PCR

components as described earlier [19]. The PCR reaction was as follows: an initial denaturation at 95°C for 5 min, with 30 cycles of denaturation at 95°C for 30s, annealing at 55°C for 30s, extension at 72°C for 60s and final extension at 72°C for 10 min. PCR products were visualized on 1% agarose gel with ethidium bromide dye under UV Doc system.

Statistical analysis Categorical variables were compared by means of either $\chi 2$ analysis or Fisher's exact test when needed. A 2-tailed P value of <0.5 was considered significant. All statistical calculations were done using standard programs using statistical package for social sciences (SPSS) 18 software.

RESULTS

Among the total 151 *S.aureus* isolates randomly selected from previous eight years and processed for complete re-identification, all isolates were positive for catalase, coagulase and DNase tests. All isolates scored positive for the *nuc* gene on PCR with an expected size of 270 bp, while *mecA* gene was revealed by PCR with an expected size of 533bp in 54 (35.8%) isolates (considered as MRSA), and the remaining 97 (64.2%) isolates were identified MSSA.

Table 1 depicts the results obtained on disk agar diffusion assay. All *S.aureus* isolates were found to be sensitive to teicoplanin. Vancomycin sensitivity tested by MIC assay revealed 98.6% isolates to be susceptible towards this antibiotic with MIC being <2µg/ml, while other isolates showed intermediate level susceptibility (MIC ranged from 4-8µg/ml) , the so called Vancomycin intermediate *S.aureus* (VISA).

Of the total isolates of *S.aureus*, 47 (31.1%) were found as MDR, of which 10 (21.3%) of them were MSSA and 37 (75.5%) MRSA. Of these MDR isolates, 34 (72.3%) were resistant to azithromycin, 34 (72.3%) to ceftriaxone, 35 (74.5%) to ciprofloxacin, 34 (72.3%) to trimethoprim-sulphamethaxazole, 32 (68%) to gentamicin, and 41 (87.2%) to penicillin. Also, 7 of the 9 imipenem resistant isolates, all ofloxacin (n=12), linezolid (n=9), rifampin (n=3) and two vancomycin resistant isolates were MDR. Interestingly, all MDR strains were sensitive to teicoplanin, while only 3 of them were resistant to meropenem.

Resistance to pattern "CRO, AZM, CIP, SXT, GM, OX, P, CC, E" was observed in 10 (21.3%) of the isolates.

A meaningful relation was observed when the number of MDR isolates and the years of isolation was compared ($p < 0.05$). In year 2007, 33.3% of isolates were found as MDR, while this trend increased to 87.5% in 2014. No relationship was observed between the years of isolation and resistance to any single antibiotics, or the source of isolation and antibiotic resistance.

Of the total isolates, 137 (91.4%) were positive for at least one SEg gene, while none of the 18 genes encoding virulence determinants, was detected in 14 isolates. The most frequent genes namely *sea, sek, sep* and *seq*, coding for enterotoxins A, K, P and Q respectively, were harbored by eight isolates. In other isolates (n= 129; 94.16%) more than one gene was present in the same isolate. Whereas no isolate harbored *sej* and *see* genes, which code for enterotoxins J and E respectively, occurrence of other genes was found in the following declining order: *sea*: 87 (57.6%), *sek*: 77 (51%), *seq*: 69 (45.7%), *seg*: 64 (42%), *seo*: 57 (37,7%), *sei*: 55 (36.4%), *sem*: 55 (36.4%), *sen*: 46 (30.5%), *sec*: 34 (22.5%), *seu*: 34 (22.5%), *sep*: 14 (9.3%), *sel*: 9 (6%), *sed*: 8 (5.3%), *seh*: 7 (4.6%), *seb*: 2 (1.3%), and *ser*: 2 (1.3%) .

Of the total 137 *S.aureus* isolates harboring SE genes, 26 (18.98%) were found to harbor the five common *egc* 1 genes (*seg-sei-sem-sen-seo*), the so called "complete" locus or *egc*1 locus. The *egc 2* gene combination, consisting of six genes (*seg-sei-sem-sen-seo-seu*) was observed in 13 (9.49%) of the isolates. The most common toxin genotype was *sea-sek-seq* (10.95%), followed by *sek-seq* (8.76%).

When frequency of enterotoxin genes was compares in relation to various clinical samples, *S.aureus* isolated from blood and wound harbored enterotoxin genes namely, *sea, sek, seq, sei, sem* and *seo* in combination more frequently as compared to other enterotoxin genes. Isolates obtained from urine also possessed *sea* gene combination more frequently than other gene combinations (Table 2).

MRSA and MSSA isolates differed in the distribution of enterotoxin genes. The most common toxin genotype in MRSA was *sea-sek-seq* (9.93%) which comprises 24% of the MRSA isolates, making it the most common genotype

in MRSA strains, followed by *sek-seq* (6.62%) which comprises 16% of the MRSA isolates, however their presence was not meaningfully associated with methicillin resistance. Only one MRSA isolate did not contain any SEg genes.

Table 2 Single and in combination possession of enterotoxin genes by *S.aureus* with respect to various clinical samples

Status of possession of genes	Isolates (n=137)									
	Total No. of isolates (%)	Blood	Urine	Wound	Sputum	Syno-vial fluid	Abscess	Bile	Endo-tracheal	Catheter
Single										
sea	1 (0.7)	1	-	-	-	-	-	-	-	-
sek	3 (2.2)	-	1	2	-	-	-	-	-	-
seq	2 (1.5)	-	-	2	-	-	-	-	-	-
sep	2 (1.5)	-	-	1	1	-	-	-	-	-
Combination										
sea	86(62.77)	30	12	30	3	4	1	-	4	1
sek	69(50.36)	24	11	27	2	3	1	1	3	2
seq	65 (47.4)	28	7	22	2	1	1	1	3	2
sep	12 (8.8)	2	2	7	1	-	-	-	-	-
seg	64 (46.7)	27	5	25	2	2	-	1	1	1
sei	55 (40.1)	22	4	20	3	3	-	1	1	1
sem	55 (40.1)	25	5	20	2	2	-	-	-	1
sen	46 (33.6)	21	5	18	1	-	-	1	-	-
seo	57 (41.6)	25	5	20	2	2	-	-	2	1
seu	34 (24.8)	20	-	10	-	2	-	-	1	1
sed	8 (5.8)	2	-	5	-	-	-	-	-	-
seb	2 (1.5)	2	-	-	-	-	-	-	-	-
sec	34 (24.8)	9	8	11	1	2	-	-	2	1
sel	9 (6.6)	2	3	3	-	-	-	-	-	1
see	-	-	-	-	-	-	-	-	-	-
seh	7 (5.1)	2	1	4	-	-	-	-	-	-
sej	-	-	-	-	-	-	-	-	-	-
ser	2 (1.5)	-	-	2	-	-	-	-	-	-

Table 3 *agr* types in relation with methicillin resistance

agr types		Methicillin resistance		
		MSSA	MRSA	Total
	agr1	64	51	115
	agr2	2	0	2
	agr3	21	2	23
	agr4	4	0	4
	Total	91	53	144

In 97 MSSA isolates, the most common genotype was *sea- sec- sek- seq* (4.1%). Of the 14 strains with no SEg genes 13 (92.9%) were MSSA strains. Enterotoxin C, I, P and U were observed nearly two times in MSSA over MRSA isolates. Frequency of *seg, sem* and *seo* genes was almost four times in MSSA isolates than MRSA ones, while *seh* did not differ in both type of isolates. Enterotoxin genes *sei* and *sel* were harbored nearly three times and *sen*, five times in MSSA and MRSA isolates respectively. The only gene possessed by MRSA isolates, though in low frequency was *seb*, while on the other hand *ser* was observed in only MSSA isolates.

When a multiplex PCR test using one pan forward primer and four reverse primers for the four *agr* types was conducted, 144 (95.4%) of 151 clinical *S.aureus* isolates could be typed into four *agr* types. A total of 115 (76.2%) out of 144 isolates were of *agr* type I, the other 23 (15.2%) being *agr* type III, 4 (2.6%) isolates being *agr* type IV and 2 (1.3%) isolates being *agr* type II. Seven (4.6%) isolates could not be typed by *agr* typing.

Certain genotypes were closely associated with specific *agr* types for example, genotype *sek, seq* being associated with *agr* type 1 (92%), genotype *sea, sek, seq* and genotype *sea sec, sek, seq* with *agr* type 1 (100%) and the *egc* genotype (*seg, sei, sem, sen, seo, seu*) with *agr* type 3 (80%).

agr types in association with methicillin resistance is demonstrated in Table 3. A meaningful relationship was observed between *agr* types and methicillin resistance ($p < 0.05$). Almost 94% of MRSA isolates belonged to *agr* group I.

Table 4 Frequencies of different SEg genes, *mecA* genes and *agr* groups in each year

year	2007				2008				2009				2010				2011				2012				2013				2014				Total***	
agr	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
P*	73	8	8	8	83	0	16	0	78	0	7	14	77	0	22	0	82	0	17	0	81	0	18	0	100	0	0	0	100	0	0	0		
R**	100	0	0	0	100	0	0	0	100	0	0	0	95	0	5	0	75	0	25	0	100	0	0	0	100	0	0	0	100	0	0	0		
sea	10	-	1	2	5	-	1	-	5	-	1	-	20	-	8	-	10	-	3	-	7	-	1	-	4	-	-	-	6	-	-	-	57.6	
seb	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	
sec	7	-	1	2	1	-	-	-	1	-	-	-	7	-	2	-	2	-	-	-	3	-	1	-	1	-	-	-	3	-	-	-	22.5	
sed	1	1	-	1	-	-	-	-	-	-	-	-	4	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.3	
see	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
seg	6	-	2	2	5	-	1	-	2	-	1	2	12	-	8	-	9	-	2	-	4	-	1	-	2	-	-	-	3	-	-	-	42.4	
seh	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	4.6	
sei	6	-	2	2	3	-	1	-	1	-	-	2	11	-	7	-	8	-	3	-	4	-	-	-	2	-	-	-	-	-	-	-	36.4	
sej	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
sek	13	2	1	-	5	-	1	-	8	-	-	1	15	-	1	-	8	-	-	-	6	-	1	-	5	-	-	-	7	-	-	-	51	
sel	3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	6	
sem	4	-	1	1	3	-	2	-	1	-	1	1	9	-	8	-	8	-	3	-	3	-	1	-	2	-	-	-	3	-	-	-	36.4	
sen	5	-	1	2	4	-	1	-	1	-	-	2	8	-	6	-	8	-	2	-	2	-	1	-	1	-	-	-	2	-	-	-	30.5	
seo	4	-	1	1	3	-	2	-	1	-	1	1	11	-	8	-	8	-	3	-	3	-	1	-	2	-	-	-	3	-	-	-	37.7	
sep	2	-	-	-	2	-	-	-	-	-	-	-	3	-	-	-	1	-	1	-	2	-	-	-	1	-	-	-	-	-	-	-	9.3	
seq	9	1	-	-	6	-	-	-	6	-	-	-	19	-	3	-	4	-	2	-	5	-	-	-	6	-	-	-	7	-	-	-	45.7	
ser	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	
seu	1	-	2	2	-	-	1	-	-	-	1	1	8	-	7	-	1	-	3	-	-	-	-	-	2	-	-	-	3	-	-	-	22.5	
*mec*A	10	-	-	-	5	-	-	-	5	-	-	-	15	-	1	-	3	-	1	-	3	-	-	-	4	-	-	-	6	-	-	-	35.8	

** Percentage of agr type in each year*
*** Percentage of each agr group for methicillin resistance (mecA positive) in each year*
****Prevalence among strains*

Another interesting feature observed in our study was the steady increase in *agr*I isolates during eight years (from 2007- 2014) (Table 4). *agr* groups II, III and IV became less prevalent and *agr* group I became dominant type and interestingly, all the *S.aureus* investigated in 2014 were from *agrI* group. The prevalence of *S.aureus* in various *agr* groups in each year was as follows:

Year 2007: *agrI* (73), *agrII* (8), *agrIII* (8) and *agrIV* (8); Year 2008 *agrI* (83), *agrIII* (16); Year 2009 *agrI* (78), *agrIII* (7) and *agrIV* (14); Year 2010 *agrI* (77), *agrIII* (22); Year 2011 *agr I* (82), *agrIII* (17); Year 2012 *agrI* (81), *agrIII* (18); Year 2013 *agrI* (100); Year 2014 *agrI* (100)

DISCUSSION

The present investigation revealed resistance of *S.aureus* isolates towards penicillin, and gradual increase in prevalence of methicillin resistance in *S.aureus* isolates during eight year period of time. Since prevalence of MRSA attributes more to development of resistance to other antibiotics too, this is a therapeutic concern. These results were compatible with other investigators [8, 21] and our previous study [17]. The disparity with our previous study is the higher frequency of VISA strains found in year 2013, while in the present investigation, since not all strains were enrolled and random sampling was done thus, only two strains were found as VISA. Teicoplanin was the only 100% effective antibiotic, maintaining its status as one of the best antibiotics against MRSA isolates. More than 50% resistance to methicillin is reported in most tertiary care centers [1], while in Iran relative frequency of MRSA on an average has been reported to be 52.7% [2]. Surprisingly, we found much lower prevalence (35.8%) on an average. This is due to the isolates being utilized from previous years. A study from South of India [30] disclosed 31.1% MRSA isolated from clinical samples, of which 63.6% were MDR. In another study, 60% of *S. aureus* isolates were found as MRSA and almost 50% of them were resistant to antibiotics of the first line of therapy [9]. Although cases of VISA have been reported from US and Japan, resistance to vancomycin is very low in Iran [17]. A similar condition is observed in neighboring countries like Pakistan [8] and North of India [3] whereby, 3.3% VISA were reported. Our study found rifampin, imipenem and meropenem among the effective antibiotics against *S.aureus.*

As regards to the numerous staphylococcal super antigen genes, eighteen SEg genes were investigated in this study. These genes are carried on mobile genetic elements (MGEs) along with other virulence and resistance genes, and they are associated with particular lineages [18, 19, 24] Though few studies are available on super antigens or on the presence of SE in humans isolates of *S.aureus* [4, 20] or in animal isolates [16, 36, 37], there exist lacunae in this regard from Iran. Herein, 90.7% of *S.aureus* isolates collected over an 8-year period revealed 57.6% had at least one

classical SE gene. Of the total isolates studied, 18.9% possessed the complete *egc* locus (*selo*, *selm*, *sei*, *seln*, *seg*) as determined by both multiplex PCR and the use of primers for single genes. The other genes were less frequent comparatively. The most common SEg gene observed was *sea* (57.6%), followed by *sek* (45.7%) and *seq* (51%) which is compatible with results of other studies [19, 40]. Among all enterotoxin genes studied, *sea* followed by *sek* and *seq* were much more prevalent among MRSA (24%) than MSSA (4%).

The concept introduced by Falkow [15] attributes polygeny and mobility of virulence genes to emergence of a "virulent clone". The present study followed this concept , however, a meaningful relation could not be found when prevalence of SE genes in MRSA or MSSA isolates or a particular infection was compared, however, the most common SE genes in MRSA strains were observed to be *sek*, *seq* and *sea*. Among the SE genes, *seb* was present only in MRSA strains and SE gene *ser* was the gene present only in MSSA strains. The other remarkable outcome was the absence of *see* and *sej* genes in our isolates in contrast to the findings of other studies performed on SE genes [35]. The number of different combinations found indicates that the genetic diversity in *S.aureus* is high in the isolates.

The findings of studies conducted in Germany, Japan, Bulgaria, Poland and New Zealand, in which nasal carriage isolates were screened for 12 to 18 of the SEgs, revealed that 75 to 100% percent of the *S.aureus* isolates harbored at least one SE gene. In different countries, different SE genes are predominant; for example studies from Ireland, New Zealand and Japan report *seb*; Poland, *sec*; Germany, *sea*, and *sec*; Bulgaria, *sea* [7, 11, 12, 27, 28] as the most common SE genes. We found *sea* to be the predominant type. Possession of *egc* loci in *S.aureus* isolates in above studies is compatible with our research study. Variations in the results of different studies points towards utilizing different study populations (healthy individuals or patients), different PCR procedures, amplification cycles, primers, single or multiplex PCR, or even using various brands of thermocyclers [7, 10, 22, 33, 34]. Some varieties have also been observed regarding DNA sequences of *egc* alleles which affects primer binding to the genes and thus limits the ability of PCR in detecting *egc* genes [7, 26].

Study conducted on *S.aureus* virulence determinants [22] disclosed presence of all four *agr* types to be distributed evenly in *S.aureus* clinical isolates. In another study [38] *agr* group I was the most prevalent among 192 carrier and disease isolates, and 71% of these isolates were MRSA strains, which are known to be highly clonal. In the present investigation, irrespective of span of eight year period, more than 75% *S.aureus* isolates belonged to *agr* type I. A close relation between methicillin resistance, dominant toxin genotypes and *agr* groups (94.4% of MRSA strains with *sea- sek- seq* genes belonged to *agr* group I) was observed. These findings suggest that MRSA is being pushed toward becoming the dominant form of *S.aureus* through bacterial interference by means *agr* locus.

We infer that maintenance of almost similar genetic elements (*sea, sek* and *seq* (in decreasing order) coding for enterotoxins A, K and Q respectively) during past eight years, predicates better epidemiological situation. However, exhaustive sampling is needed to establish any concrete conclusion. Emergence of isolates possessing a certain spectrum of virulence genes along with isolates of the similar *agr* (*agr*I) and *egc* (*egc* I) type may be a worrying situation, especially in relation to the increasing frequency of MRSA strains.

Acknowledgement
This is a report of a database from MSc thesis (Grant No.90/2-2/3) of Mr. Milad Sabaee registered in Infectious Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. The authors wish to thank Dr. Behrouz Naghili and Dr. Mojtaba Varshochi for their constant support.

REFERENCES

[1] Arakere G, Nadig S, Swedberg G, Macaden R, Amarnath S K, Raghunath D, *J Clin Microbiol*, **2005**, 43, 3198-3202
[2] Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, Nasab MN, *Iranian J Basic Med Sci*, **2012**, 15, 1010-1019
[3] Assadullah S, Kakru D K, Thoker M A, Bhat F A, Hussain N, Shah A, *Indian J Med Microbiol*, **2003**, 21, 196-8
[4] Aucken H M, O'Neill G, Ganner M, Dinerstein N, Ali M, Murchan S, *J Hosp Infect*, **2006**, 63, 170–178
[5] Bachert C, Gevaert P, Cauwenberge P V, *Curr Allergy Asthma Report*, **2000**, 2, 252–258
[6] Balaban N, Novick R P, *Proc. Natl. Acad. Sci. USA*, **1995**, 92,1619–1623

[7] Bania J, Dabrowska A, Korzekwa K, Zarczynska A, Bystron J, Chrzanowska J, Molenda J, *Lett Appl Microbiol*, **2006**, 42, 315–320
[8] Bano S, Tunio S A, Mal S, Jatt A N, *Sindh Univ. Res. Jour.* (Sci. Ser.) **2012**, 44 (4), 683-686
[9] Baveja S, De A, Taklikar S, Sonavane A, Wanjari K, *Journal of Evolution of Medical and Dental Sciences*, **2012**, 6(1), 944-951
[10] Beavis R, Novick R P, Ji G, *Science*, **1997**, 276, 2027–2030.
[11] Becker K, Friedrich A W, Lubritz G, Weilert M, Peters G, Von Eiff C, *J Clin Microbiol*, **2003**, 41, 1434–1439
[12] Boerema J A, Clemens R, Brightwell G, *Int J Food Microbiol*, **2006**, 107, 192–201
[13] Collery M M, Smyth D S, Twohig J M, Shore A C, Coleman D C, Smyth C J, *J Med Microbiol*, **2008**, 57, 348-358
[14] Dinges M M, Orwin P M, Schlievert P M, *Clin Microbiol Rev*, **2000**, 13,16–34
[15] Falkow S, *ASM News*, **1997**, 63,359-365
[16] Fitzgerald J R, Hartigan P J, Meaney W J, Smyth C J, *J Appl Microbiol*, **2000**, 88, 1028–1037
[17] Hasani A, Sheikhalizadeh V, Hasani A, Naghili B, Valizadeh V, Nikoonijad A R, *Iranian J Microbiol*, **2013**, 5,56-62
[18] Holtfreter S, Broeker B M, *Arch. Immunol. Ther. Exp*, **2005**, 53,13–27
[19] Holtfreter S, Grumann D, Schmudde M, Nguyen H T T, Eichler P, Strommenger B, Kopron K, Kolata J, Giedrys-Kalemba S, *J Clin Microbiol*, **2007**, 45, 2669–2680
[20] Humphreys H, Keane C T, Hone R, Pomeroy H, Russell R J, Arbuthnott J P, Coleman D C., *J Med Microbiol*, **1989**, 28, 163–172
[21] Janapatla R P, Yanb J J, Huang A H, Chenb H M, Wu H M, Wu J J, *Diag Microbiol Infect Dis*, **2007**, 58(2), 203-209
[22] Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, Nesme X, Etienne J, Vandenesch F, *Infect. Immun*, **2002**, 70, 631–41.
[23] Lebeau C, Vandenesch F, Greenland T, Novick R P, Etienne J, *J. Bacteriol*, **1994**, 176,5534-36
[24] Lindsay J A, Holden M T, *Integr Genomics*, **2006**, 6,186–201
[25] Mayville P, Beavis R, Ji G, Yang H, Goger M, Novick R P, Muir T W, *Proc. Natl. Acad. Sci. USA*, **1999**, 96,1218–1223
[26] Monday S R, Bohach G A, *J Clin Microbiol*, **1999**, 37, 3411–3414
[27] Nashev D, Toshkova K, Bizeva L, Akineden O, Lammler C, Zschock M, *Lett Appl Microbiol*, **2007**, 45, 681–685
[28] Omoe K, Hu D L, Takahashi-Omoe H, Nakane A, Shinagawa K, *FEMS Microbiol Lett*, **2005**, 246, 191–198
[29] Peng H L, Novick R P, Kreiswirth B, Kornblum J, Schlievert P, *J. Bacteriol*, **1988**, 170, 4365-4372
[30] Rajaduraipandi K, Mani K R, Panneerselvam K, Mani M, Bhaskar M, Manikandan P, *Indian J Med Microbiol*, **2006**, 24, 34-8
[31] Read A F, Taylor L H (**2001**) *Science* 292,1099–1102.
[32] Rushdy A A, Salama M S, Osman A S, *International Journal of Agriculture & Biology*, **2007**, 9 (6), 800-806
[33] Saunders G C, Dukes J, Parkes H C, Cornett J H, *Clin Chem*, **2001**, 47, 47–55
[34] Schoder D, Schmalwiesser A, Schauberger G, Hoorfar J, Kuhn M, Wagner M, *J Clin Microbiol*, **2005**, 43, 2724–2728.
[35] Sila J, Sauer P, Kolar M, *Comparison of the prevalence of genes coding for enterotoxins, exfoliatins, panton-valentine leukocidin and tsst-1 between methicillin-resistant and methicillin-susceptible isolates of Staphylococcus aureus at the university hospital in Olomouc.* Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, **2009**, 153(3), 215-8
[36] Smyth D S, Hartigan P J, Meaney W J, Fitzgerald J R, Deobald C F, Bohach G A, Smyth C J, *J Med Microbiol*, **2005**, 54, 401–411
[37] Smyth D S, Kennedy J, Twohig J, Miajlović H, Bolton D, Smyth C J, *J Food Prot*, **2006**, 69, 508–515
[38] Van Leeuwen W, Van Nieuwenhuizen W, Gijen C, Verbrugh H, Van Belkum A, *J. Bacteriol*, **2000**, 182, 5721-5729
[39] Wayne P A. Clinical and Laboratory Standards Institute/NCCLS, Performance Standards for Antimicrobial Susceptibility Testing. Fifteenth Informational Supplement, **2014**, CLSI/NCCLS document M100-S15. CLSI
[40] Wu D, Li X, Yang Y, Zheng Y, Wang C, Deng L, Liu L, Li C, Shang Y, *J. Med. Microbiol*, **2011**, 60, 35-45