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# Prevalence and Distribution of Superantigen Toxin Genes in Clinical Community Isolates of *Staphylococcus Aureus*

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## Abstract

To investigate the distribution of twelve staphylococcal enterotoxin genes (*se*) and the toxic shock syndrome toxin-1 gene (*tst*) in *Staphylococcus aureus*, 140 community isolates from various origins were investigated. Isolates were collected, from 15 clinical laboratories located at Casablanca, between 2007 and 2008, they were identified by conventional methods, and methicillin resistance was confirmed by amplification of *mec*A gene by PCR. All isolates were searched using a multiplex PCR for the accessory gene regulator (*agr*) group, and for thirteen superantigen (SAg) toxin genes: *sea, seb, sec, sed*, *seh, selk, sell, selm, selo, selp, selq*, *ser* and *tst*.

Among all isolates, only two were methicillin-resistant and one hundred seven were shown to be positive for at least one of the tested SAg toxin genes. They were grouped in 43 genotypes. Our work showed that *agr* group III and *agr* group I *S. aureus* isolates, were highly prevalent for the presence of *seh*, *selq*, *selk* and/or *tst* genes, on one hand, and *sec* and/or *sell* genes, on the other hand (P<0.05), respectively. In addition, we found a relationship between pus/wound *S. aureus* isolates and the presence of *selk* + *selq* genes (P<0.05).

Our results suggest that *agr* group III isolates carried more of SAg toxin genes than *agr* groups I and II *S. aureus* strains.

**Keywords:** *Staphylococcus aureus*; MSSA; MRSA; SAgs toxin genes; *agr* groups; Community

## Introduction

*Staphylococcus aureus* is a major cause of multiple types of infections both in and outside of the hospital setting. These infections range from superficial skin infections to deeper infections of hair follicles, abscesses, and deep tissue infections, and even to systemic infections including those of the heart, lungs, bones, and blood [1].

The organism has an array of cell-surface and secreted virulence factors that allow it to cause illnesses [1]: the surface virulence factors allow *S. aureus* to colonize the host, through adhesion to mucosal surfaces and resistance to phagocytosis, the secreted factors, including exoenzymes and exotoxins, allow the organism to interfere with normal immune system function, spread into surrounding tissues, and access nutrients through cell damage. Among the secreted virulence factors that have known roles in serious human diseases, are the staphylococcal superantigens (SAgs) [2,3]. They includes toxic shock syndrome toxin-1 (TSST-1) [4], staphylococcal enterotoxin (SE) serotypes A, B, Cn (in which n denotes that multiple variant forms exist), D, E, G, H ,I , R, S, T and SE-like (SE*l*s) serotypes J, K, L, M, N, O, P, Q, U, V [5-7]. These toxins are considered to be major virulence factors of *S. aureus* [3,5]. In addition, they exhibited superantigen activity, stimulating polyclonal T-cell proliferation through colligation between major histocompatibility complex class II molecules on antigen-presenting cells (APC) and the variable portion of the T-cell antigen receptor ß chain or α chain (TCR Vß and TCR Vα, respectively), with no need for prior APC processing [2,8,9]. On the other hand, most of genes encoding these toxins are located on mobile genetic elements, such as bacteriophages, pathogenicity islands (SaPIs), genomic islands, and plasmids. This association implies a horizontal transfer of the SAg toxin genes between staphylococcal strains and an important role in the evolution of *S. aureus* as a pathogen. [10-14].

However, expression of most virulence genes in *S. aureus* is controlled by the accessory gene regulator (*agr*) locus. The *agr* locus consists of two divergent transcription units driven by promoters P2 and P3. The P2 operon encodes a two component signalling module, AgrC is the receptor and AgrA is the response regulator. It also encodes two proteins, AgrB and D, witch combine to produce and secrete an autoinducing peptide (AIP) that is the ligand for AgrC. AgrA functions to activate transcription from its own promoter and from the *agr*P3 promoter, which drives the synthesis of RNAIII, the effector of target gene regulation [15]. Sequence variation in *agr*B, *agr*D and *agr*C has led to the identification of at least four *S. aureus agr* specificity groups (I to IV) [16]. Furthermore, the Agr system has been assigned a central role in *S. aureus* pathogenesis [13,17,18].

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Little information is available about the degree of superantigen genetic variability among populations of community clinical *Staphylococcus aureus* in Morocco. For this reason, the purpose of the present study was to investigate the presence of the staphylococcal superantigen toxin genes in a group of *S. aureus* isolates in the city of Casablanca (Morocco) and to correlate them with their origin, and *agr* groups.

## Materials and Methods

### Bacterial isolates

Non-duplicate community *S. aureus* strains from clinical specimens were collected from outpatients of the Laboratory of Microbiology, Institute Pasteur of Morocco, and from 14 clinical laboratories located in Casablanca, between January 2007 and October 2008. Patients were assessed as to whether they had previously been hospitalised for a medical condition.

### Identification of *S. aureus* isolates

Species were identified by colony morphology, gram staining, catalase test, coagulase activity on rabbit plasma (Bio-Mérieux, Marcy l'Etoile, France), and production of clumping factor (Pastorex Plus-Staph, Bio-Rad, Marnes-la-Coquette, France).

### Antimicrobial resistance

Antimicrobial resistance to penicillin G, kanamycin, tobramycin, gentamicin, tetracycline, erythromycin, lincomycin, pristinamycin, chloramphenicol, pefloxacin, fosfomycine, cefoxitin, fusidic acid, rifampicin, vancomycin and trimethoprim-sulfamethoxazole was determined by the standard disc (Bio-Rad, Marnes-la-Coquette, France) diffusion technique. Results were interpreted according to the Committee for Antimicrobial Testing of the French Society of Microbiology guidelines (http://www.sfm.asso.fr) (Comité de l'Antibiogramme de la Société Française de Microbiologie, 2007). *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used as quality control organisms.

### DNA extraction

All isolates of *S. aureus* were grown in brain heart infusion media at 37°C overnight. Their genomic DNA used for polymerase chain reaction (PCR) was extracted by using a standard phenol-chloroform procedure as described by Sambrook et al., [19]. Amplification of *nuc* gene which encodes an extracellular thermo stable nuclease of *S. aureus* was used, as described by Brakstad et al., [20], for identification and to confirm the quality of each DNA extract. Strains with phenotypic resistance to methicillin were confirmed by polymerase chain reaction detection of the *mec*A gene as described by Vannuffel et al. [21]. Two reference strains: U2A1593 for methicillin-resistant *S. aureus* (MRSA) and U2A1594 for methicillin-sensitive *S. aureus* for (MSSA) from the Antibacterial Agents Unit of Pasteur Institute, Paris, France, were used as controls.

### Detection of staphylococcal toxin genes

Several Multipex PCRs for the parallel detection of the presence of the following genes were performed: the classical staphylococcal enterotoxins (*sea, seb, sec* and *sed*), SEs and SE*l*s (*seh, selk, sell, selm, selo, selp, selq* and *ser*) and the toxic shock syndrome toxin-1 (*tst*) [16,22]. *S. aureus* ATCC19095 (*sec, seh, sell, seg, sei, selm, seln, selo* and *seu*); FRI913 (*sea, sec, see, selk, sell, selq* and *tst*); ATCC14458 (*seb*); were used as positive control strains. Control chromosomal DNA samples for *sed*, *ser* and *selp* genes were obtained from our standard laboratory controls.

### Determination of *agr* groups

A multiplex PCR of the *agr* was used to determine the *agr* group (I-IV) [23]. *S. aureus* strains RN6390 (*agr* group I), RN6607 (*agr* group II), RN8465 (*agr* group III), and RN4850 (*agr* group IV) from the National Research Center of Lyon (CNR-Lyon, France) were used for *agr* group identification.

### Statistical analysis

Chi-square test was used to study the correlation between the prevalence of the SAg toxin genes and the *agr* group of *S. aureus* isolates on one hand, and the prevalence of these SAg toxin genes and the origin of *S. aureus* isolates on the other hand. Statistical analysis of the data was performed on SPSS. $P < 0.05$ was considered statistically significant.

| ***agr* group (n,%)** | | **No of *S.aureus* isolates with virulence SAg gene: (*genotype, n*)** | | | | | **Total SAg genes, n (%)** |
|---|---|---|---|---|---|---|---|
| | **None** | **One** | **Two** | **Three** | **Four** | **Five** | |
| **I (66, 47.2)** | 23 | *seb,1* | *seb sep, 1* | *seb sem seo, 2* | *sec sel sem seo, 9* | *seb sec sel sem seo, 1* | |
| | | *sec, 1* | *sec sep, 1* | *sec sel seo, 3* | | *sec sel sem seo tst, 1* | |
| | | *sel, 1* | *sec sem, 1* | *sek sep seq, 1* | | | |
| | | *sem, 1* | *sem seo, 12* | *sem seo sep, 1* | | | |
| | | *seo, 3* | | *sem seo tst, 1* | | | |
| | | *sep, 2* | | | | | |
| **Sous total of *SAg genes/ agrI* (%)** | **23 (34.9)** | **9 (13.7)** | **15 (22.7)** | **8 (12.1)** | **9 (13.6)** | **2 (3.0)** | **43 (65.15)** |
| **II (29,20.7)** | 10 | *seo, 1* | *seb sep, 1* | *sec sem seo, 1* | *sec sel sem seo, 1* | *sed sem seo sep ser, 1* | |
| | | *tst, 1* | *sem seo, 7* | *sem seo sep, 4* | *sed sem seo ser, 1* | | |
| | | | *sem tst, 1* | | | | |
| **Sous total of *SAg genes/ agrII* (%)** | **10 (34.5)** | **2 (6.9)** | **9 (31.0)** | **5 (17.3)** | **2 (6.9)** | **1 (3.4)** | **19 (65.51)** |
| **III (42,30.0)** | 0 | *seh, 4* | *sea seh, 1* | *sec seo tst, 1* | *sea sec seh sel, 1* | *sec seh sek sel seq, 1* | |
| | | *seo, 3* | *seh seo, 1* | *sec seh sek, 1* | *sea seh sek seq, 1* | | |
| | | | *sek seq, 2* | *seh sek seq, 3* | *seb seh sek seq, 1* | | |
| | | | *sem seo, 7* | *seh seo tst, 1* | *sec seh sek seq, 1* | | |
| | | | *seo tst, 2* | *sek sem seo, 1* | *seh sek seo seq, 1* | | |
| | | | *ser tst, 1* | *sem seo tst, 5* | *seh sek seq tst, 2* | | |
| | | | | | *seh seo ser tst, 1* | | |
| **Sous total of *SAg genes/ agrIII* (%)** | **0** | **7 (16.7)** | **14 (33.3)** | **12 (28.6)** | **8 (19.0)** | **1 (2.4)** | **42 (100.00)** |
| **IV (3, 2.1)** | 0 | | *sem seo, 2* | *seb sel seo, 1* | 0 | 0 | |
| **Sous total of *SAg genes/ agrIV* (%)** | **0** | **0 (0.0)** | **2 (66.7)** | **1 (33.3)** | **0** | **0** | **3 (100.00)** |
| **Sous total of *SAg genes/ agr* I, II, III,IV** | **33** | **18** | **40** | **26** | **19** | **4** | **107 (76.4)** |

N.B: *sek, sel, sem, seo, sep* and *seq* genes = *staphylococcal enterotoxin-like*

**Table 1:** Combination of SAg genes in clinical isolates of *S. aureus* according to their *agr* group (n=140).

| Genes | No. (%) of isolates from: (n=140) | | | | | P value |
|---|---|---|---|---|---|---|
| | genital-urinary tract[b] (n=57) | pus/wound (n=52) | sputum (n=16) | naso-pharynx[c] (n=13) | blood (n=2) | |
| ***sea*** | 2 (3.5) | 1 (1.9) | - | - | - | 1.000 |
| ***seb*** | 4 (7.0) | 3 (5.8) | 1 (6.3) | - | - | 1.000 |
| ***sec*** | 8 (14.0) | 9 (17.3) | 3 (18.9) | 3 (23.1) | 1 (50.0) | 0.837 |
| ***sed*** | - | 2 (3.8) | - | - | - | - |
| ***seh*** | 7 (12.3) | 10 (19.2) | 2 (12.6) | 1 (7.7) | - | 0.463 |
| ***sek*** | 2 (3.5) | 9 (17.3)[a] | 2 (12.6) | 2 (15.4) | - | 0.038 |
| ***sel*** | 6 (10.5) | 6 (11.5) | 2 (12.6) | 2 (15.4) | 2 (100.0) | 1.000 |
| ***sem*** | 22 (38.6) | 23 (44.2) | 9 (56.3) | 6 (46.2) | 1 (50.0) | 0.688 |
| ***seo*** | 27 (47;7) | 32 (61.5) | 9 (56.3) | 6 (46.2) | 1 (50.0) | 0.197 |
| ***sep*** | 8 (14.0) | 3 (5.8) | 1 (6.3) | - | - | 0.266 |
| ***seq*** | 2 (3.5) | 8 (15.4)[a] | 1 (6.3) | 2 (15.4) | - | 0.045 |
| ***ser*** | 1 (1.75) | 3 (5.8) | - | - | - | 0.546 |
| ***tst*** | 6 (10.5) | 5 (9.6) | 2 (12.6) | 3 (23.1) | 1 (50.0) | 1.000 |

N/B: ([a]): P<0.05
([b]): urine (n = 30), vaginal and high vaginal swabs (n = 10), urethral swabs (n =8), sperm (n =9)
([c]): nose (n = 5); pharynx (n = 8)

**Table 2:** Incidence of SAg genes in *S. aureus* isolates from various sources.

| SAg genes: (n,%) | *S. aureus isolates with agr* group (n,%) | | | | Total (%) |
|---|---|---|---|---|---|
| | I (66, 47.2) | II (29, 20.7) | III (42, 30.0) | IV (3, 2.1) | |
| ***sea*** | 0 (0) | 0 (0) | 3 (7.1) | 0 (0) | 3 (1.1) |
| ***seb*** | 5 (7.6) | 1 (3.4) | 1 (2.4) | 1 (33.3) | 8 (2.9) |
| ***sec*** | 17 (25.6)* | 2 (6.8) | 4 (9.5) | 0 (0) | 23 (8.8) |
| ***sed*** | 0 (0) | 2 (6.8) | 0 (0) | 0 (0) | 2 (0.7) |
| ***seh*** | 0 (0) | 0 (0) | 20 (47.6)* | 0 (0) | 20 (7.4) |
| ***sek*** | 1 (1.5) | 0 (0) | 14 (33.3)* | 0 (0) | 15 (5.5) |
| ***sel*** | 15 (22.7)* | 1 (3.4) | 2 (4.8) | 0 (0) | 18 (6.6) |
| ***sem*** | 29 (43.9) | 16 (55.2) | 13 (31.0) | 3 (100.0) | 61 (22.4) |
| ***seo*** | 33 (50.0) | 16 (55.2) | 23 (54.8) | 3 (100.0) | 75 (27.6) |
| ***sep*** | 6 (9.0) | 6 (20.7) | 0 (0) | 0 (0) | 12 (4.4) |
| ***seq*** | 1 (1.5) | 0 (0) | 12 (28.6)* | 0 (0) | 13 (4.8) |
| ***ser*** | 0 (0) | 2 (6.8) | 2 (4.8) | 0 (0) | 4 (1.5) |
| ***tst*** | 2 (3.0) | 2 (6.8) | 13 (31.0)* | 0 (0) | 17 (6.3) |
| **No of SAg genes/ *agr* group (%)** | **109 (40.1)** | **48 (17.6)** | **107 (39.7)** | **7 (2.6)** | **271 (100.0)** |

*P<0.05

**Table 3:** Distribution of SAg genes by *agr* group of *S. aureus* isolates (n=140).

| *agr* group | N° of *S. aureus* isolates from: | | | | | Total |
|---|---|---|---|---|---|---|
| | G- U- T [a] | Pus/wound | Sputum | Naso-pharynx[b] | Blood | |
| I | 29 | 21 | 07 | 07 | 02 | 66 |
| II | 12 | 13 | 02 | 02 | - | 29 |
| III | 15 | 17 | 06 | 04 | - | 42 |
| IV | 01 | 01 | 01 | - | - | 03 |
| Total | 57 | 52 | 16 | 13 | 02 | 140 |

([a]) G-U-T: genital urinary tract (n=57), urine (n = 30), vaginal and high vaginal swabs (n = 10), urethral swabs (n =8), sperm (n =9)
([b]): nose (n = 5), pharynx (n = 8)

**Table 4:** Distribution of *agr* group *S. aureus* isolates by origin of samples.

## Results

### Antimicrobial resistances

A total of 140 *S. aureus* isolates were collected from clinical outpatient specimens at the Laboratory of Bacteriology, Institute Pasteur of Morocco (60% of isolates), and from 14 clinical laboratories located in Casablanca (40% of isolates). The rates of resistance to antibiotics were: penicillin (90.00 %), tetracycline (30.00 %), rifampicin (14.29 %), fusidic acid (12.86 %), kanamycin (9.29%), cotrimoxazole (9.29%), erythromycin (7.86%), pefloxacin (2.14%), gentamicin (1.43%), tobramycine (1.43%), lincomycin (1.43%), chloramphenicol (0.71%) and only two isolates (1.43%) were confirmed MRSA.

### Prevalence of SAg toxin genes

All of 140 *S. aureus* isolates under study harboured the *nuc* gene. The all 13 SAg toxin genes analysed in this study were detected; the prevalence were variable from 53.6% to 1.4% (*selo* 53.6%, *selm* 43.6%, *sec* 17.1%, *seh* 14.3%, *sell* 12.9%, *tst* 12.1%, *selk* 10.7%, *selq* 9.3%, *selp* 8.6%, *seb* 5.7%, *ser* 2.9%, *sea* 2.1% and *sed* 1.4%). However no virulence toxin gene was diagnosed as positive in 33 (23.6%) isolates. Whereas 1 gene was detected in 18 (12.8%) strains, in all remaining isolates (63.6%) at least two SAg genes were present in the same isolate: Forty isolates carried two genes simultaneously, twenty-six isolates contained three genes, nineteen isolates were found with four genes and in four isolates five SAg genes were detected (Table 1). A total of 43 superantigen toxin genotypes were found, the toxin genotype with the highest incidence was *selm+selo* (20%). This pair was detected in 57 of all isolates.

### Distribution of SAg genes in *S. aureus* isolates from various sources

Regarding the origin of *S. aureus* isolates, two major locations could be distinguished: genital-urinary tract (40.7%), most of isolates from this location were from urine (n = 30), the others were from vaginal and high vaginal swabs (n = 10), urethral swabs (n =8) and sperm (n =9), the second major location was pus/wound (37.1%). In addition, two minor locations were noted: sputum (11.4%) and naso-pharynx (9.3%), strains isolated from blood were scarcely represented (1.4%) (Table 2). The most abundant genes (*selo* and *selm*) were found in 61.5% and 44.2% of pus isolates, respectively; 47.7% and 38.6% of genital-urinary tract isolates, respectively; 56.3% of respiratory tract isolates, 46.2% of naso-pharynx isolates and 50 % of blood isolates (Table 2).

### Distribution of SAg genes by *agr* group

All of *S. aureus* isolates were classified according to the four *agr* groups, 47.2% strains were found to belong to *agr* group I, 20.7% were *agr* group II, 30.0% were *agr* group III and only 2.1% of isolates were found to belong to *agr* group IV. In contrast to *agr* group I or II *S. aureus* isolates, all *agr* group III strains, were positive for at least one of the tested genes (Table 1 and Table 3). A total of 22 genotypes were detected among *agr* group III *S. aureus* isolates and 16.7%, 33.3%, 28.6%, 19.0% and 2.4% of these strains contained one, two, three, four and five SAg toxin genes, respectively. Nineteen SAg genotypes were observed among *agr* group I strains, against 10 found among *agr* group II isolates. On the other hand, 13.7%, 22.7%, 12.1%, 13.6% and 3% of *agr* group I isolates carried one, two, three, four and five SAg toxin genes, respectively. 6.9%, 31.0%, 17.3%, 6.9% and 3.4% of *agr* group II *S. aureus* isolates carried one, two, three, four and five SAg toxin genes, respectively.

The most abundant SAg toxin gene (*selo*) was carried by 50.0%, 55.2% and 54.8% of isolates belonging to *agr* group I, II and III, respectively. The second abundant SAg toxin gene, namely *sem*, was carried by *agr* group I strains with 43.9%, *agr* group II with 55.2% and *agr* group III with 31.0%.

### Distribution of *agr* group *S. aureus* isolates by origin of samples

The relationship between *agr* group *S. aureus* strains and source of isolates is summarized in (Table 4).

## Discussion

*Staphylococcus aureus* isolates collected during the present study were originated from ambulatory patients, they were collected from 15 clinical laboratories located in Casablanca; therefore, they should represent randomly selected strains. In fact, a significant number were recovered from vaginal swabs, high vaginal swabs and urine. Isolates from high vaginal swabs are usually associated with puerperal sepsis or neglected foreign bodies, and less frequently with bacterial vaginosis, and significant numbers of MRSA organisms have been found among these isolates [24,25]. While it is likely that many *S. aureus* organisms might be passive colonizers or due to contamination from the skin, some are associated with complications of pregnancy [24]. Although *S. aureus* is a rare cause of urinary tract infections, accounting for only 0.5% to 6% of all positive urine cultures [26,27], their finding is increasingly being recognized as significant, especially in patients with urinary tract catheterisation, as under-treatment or delayed treatment could lead to development of staphylococcal bacteremia [26,28,29].

It is important to discuss, even briefly, the susceptibility to the tested antibiotics of all *S. aureus* isolates. This study provides important data on current antimicrobial resistance, including methicillin-resistance, for a collection of 140 recent clinical isolates of *S. aureus* from community source in Casablanca, Morocco. We found that 90% of isolates possessed resistance to penicillin, followed by strains with resistance to one or two more antimicrobial substances. Resistance to methicillin conferred by carriage of the *mec*A gene is rather low; it is found with only two *agr* group I isolates (1.43%). Epidemiological data of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) in the countries of the Maghreb, i.e. Morocco, Algeria, Tunisia and Libya are scarce; although, recent reports suggest that Hospital-acquired MRSA epidemiology is changing, with a dramatic increase of incidences [30,31].

On the other hand, it is hypothesized that the evolution of CA-MRSA is a recent event due to the acquisition of *mec* DNA by previously methicillin-susceptible strains that circulated in the community [32]. Then, considering the relationship that might exist between MRSA and MSSA isolates, and the limited data on the prevalence and distribution of SAg toxin genes among *S. aureus* isolates in Morocco; we need more specific knowledge about the circulating *S. aureus* isolates, especially MSSA strains.

In a first step, we found that the overall rate of SAg toxin gene-positive isolates reached 76.4%. This is in agreement with results published by Hu et al., [33], they have reported that 75.7 % of the MSSA isolates tested carried a number of toxin genes, ranging from 1 to 11, with extensive variation between individual strains. In a second step, we found that differences in the occurrence of genes between pus/wound versus the genital-urinary tract, sputum or naso-pharynx isolates were not significant as demonstrated by the Chi-square test ($P>0.05$); with exception for *selk* and *selq* genes, which were slightly frequent in pus/wound *S. aureus* isolates ($P<0.05$). In a third step, we found that 47.2% of all isolates were *agr* group I, this finding concurs with results of other studies [16,34]. Thereafter, we have analysed the *agr* group specificity of all strains, and we found that there are many SAg toxin genes in *S. aureus* isolates belonging to *agr* group I, II, III and/or IV from many clinical specimens. However this distribution was not uniform among all *agr* groups isolates; we found that *agr* group III *S. aureus* isolates (n=42) carried more SAg toxin genes (mean 2.55 gene/isolate), compared to 66 *S. aureus* isolates in *agr* I and 29 *S. aureus* isolates in *agr* II (mean 1.65 gene/isolate). Secondly, we found that all *agr* group III isolates (100%) were found with at least one of the tested virulence genes, whereas 23 and 10 strains among *agr* group I and *agr* group II isolates, respectively, had none SAg toxin genes. Thirdly, our finding showed that 50% of *agr* group III isolates were found with 3 to 5 virulence toxin genes, versus, only 28.9% and 27.6% of *agr* group I and II isolates, respectively. From these data, we can deduct that *agr* group III isolates were more prevalent for the presence of enterotoxin and/or *tst* genes than *agr* groups I and II isolates.

However, *agr* group I was prevalent in all clinical specimens whatever their origin, nevertheless it was slightly higher in genital-urinary tract samples than others. Whereas, both *agr* groups II and III were dominant in *S. aureus* isolates from pus/wound specimen. These differences were not statistically significant.

Most of virulence toxin genes under study are associated with mobile genetic elements. However, the repertoire of toxin genes encoded by SaPIs seems to be specified. These elements, as well as the majority of genetic elements encoding enterotoxins, can be horizontally transferred among *S. aureus* strains. But, unlike plasmids, they cannot spread autonomously. It was shown that in the presence of certain staphylococcal phages, SaPIs are excised from the genome and encapsidated. This mechanism in thought to be responsible for the transfer of pathogenicity islands [13]. For this reason the prophage φMu50A and φN315, shown to be integrated in close proximity to the TSST-1 pathogenicity island family of *S. aureus* Mu50 and N315, respectively, are considered to be involved in the horizontal transfer of these SaPIs [35]. These data led us to define the prevalence of each of the mobile genetic elements among all 140 *S. aureus* isolates under study.

In the current work, in agreement with other [22], *selm* and *selo* genes were more frequently detected in all isolates, whatever their origin and their *agr* group. Both genes belong to the recently described enterotoxin gene cluster (*egc*) that harbours 5 to 6 genes (*seg*, *sei*, *selm*, *seln*, *selo*, and sometimes *selu*), which cluster on a staphylococcal pathogenicity island type I γSaβ [33,36,37], this cluster will be found without all of the

*egc* components [33,38-40]. Furthermore, many studies reported that some isolates contained *selo* gene with *sed*, *selj* and *ser* genes instead of the *egc* genes [40-42]; in fact, Thomas et al. [42] suggested that this phenomenon is caused when a part of *egc* is exported or inserted in the chromosome of other *S. aureus* strains intermediated by insertion sequences.

Our data on the frequency of *sec* and *sell* genes are in agreement with those previously reported [33,43,44]. It is also notable that, in the presence work, *sell* gene (n = 18) was accompanied by *sec* gene in 14 *agr* group I *S. aureus* cases. This finding agrees with the fact that both genes were demonstrated to occur together on the staphylococcal pathogenicity islands SaPIm1 and SaPIn1 [35]. However, In one *S. aureus* isolate, *sell* gene was found together with *sec* and *tst* genes, these genes are associated with another mobile genetic element, named type I SaPI4 [33]. Furthermore, we have noted that *sec* and *sell* genes were also detected, but not together, in fourteen *S. aureus* isolates. Our data suggests that the staphylococci may carry other, yet unknown, pathogenic island.

The *tst* gene is carried by a family of closely related pathogenicity islands that interact in highly specific way with certain staphylococcal phages. This gene encodes for TSST-1, which is considered to be the cause of nearly all cases of menstrual TSS, and of at least 50% of nonmenstrual cases [3,45]. About 12% of our *S. aureus* isolates were *tst*-positive, however, in contrast to what has been reported by Ji et al., [46], distribution of this virulence gene by *agr* groups provide evidence that *tst* was more frequently coupled to *agr* group III and was frequently found in combination with *selm* and/or *selo* genes, this finding is in agreement with a previous observation [32,39].

On one hand, it has been known that, unlike the horizontal transfer of virulence genes, such as *tst*, most of the toxin genes that did not demonstrate this mobility type belonged to the *egc*, which is restricted to the clonal complexes CC5 (characterized by *agr* group II) and CC30 genomic background [47]. On the other hand, since most of *tst* + *sem* and/or *seo* positive isolates were *agr* III, we assume that *tst-selm* and/or *selo* positive genes isolates were CC30.

The prevalence of *seh* gene, which is rather strictly linked to CC1 [47], is highly variable. It was not detected by Sila et al., [43]; Peck et al., [48] have reported that *seh* was more frequently detected in isolates from nasal than from blood specimens; El-Huneidi et al., [49] have reported that this SAg toxin gene was only infrequently detected in clinical isolates of *S. aureus* from Jordan; Hu et al., [33] have shown that 11/140 MSSA isolates were positive for this toxin gene. Recently, in Kuwait City, Udo et al., [34] reported that staphylococcal enterotoxin gene H was found with a frequency of 21.5% among strains, but not all *agr* group III *S. aureus* isolates. Compared to other enterotoxins, *seh* gene is non-mobile, so that clonal complex (CC1) affiliation does matter.

Two of all genes which were found prevalent from pus/wound *S. aureus* isolates (*selk*, *selq*) were found without other toxin genes, whereas one *S. aureus* strain was found with *seb+selk+selq+seh* genes, and another was found with *sea+selk+selq+seh* genes. It was demonstrated that *seb+selk+selq* and *sea+selk+selq* genes are carried by SaPI3 [50] and staphylococcal phage φ3 in *S. aureus* MW2 [14], respectively.

The *selp* gene that is encoded on prophage φN315, was shown to be linked to pathogenicity islands containing the *sec* and *sell* genes, such as SaPIm1/SaPIn1 [35]. Two out of *selp*-positive isolates were found to be negative for other enterotoxin genes and one carried *sec* without *sell* gene. Interestingly, all of *sec+sell* positive strains were found to be *selp* negative; thus the linkage between these genes does not seem obvious.

Other genes, such as *sea* and *seb*, were present significantly less frequently. The fourth classical enterotoxin gene, named *sed*, was detected in 2 (1.4%) pus *agr* group II isolates, in both cases, this virulence gene was accompanied by *ser* gene, with *selm* and *selo*. It was reported that the coexistence of *sed, selj* and *ser* genes are commonly found on plasmid pIB485 [51,52]. However, *ser* gene was also found in two other *agr* group III *S.aureus* isolates (isolated from pus and urinary tract infections), without carrying the *sed* gene.

## Conclusions

Despite the fact that the *agr* group I was the most prevalent group, among 140 community *S. aureus* isolates, in Casablanca, Morocco, strains belonging to the *agr* group III harboured more virulent superantigen toxin genes. So, we can assume that *agr* group III isolates may carry more enterotoxin and/or *tst* genes than *agr* groups I and II isolates. Of all thirteen SAg toxin genes, two were detected more frequently in all *agr* group isolates, whatever their origin: *selo* and *selm*.

Statistical analysis of the comparison of the prevalence of SAg toxin genes in the four studied *agr* group isolates (*agr* group I, II, III and VI), on one hand, and the prevalence of these virulence toxin genes according to the source of isolates, on the other hand, using the chi-square test, showed that *agr* group III and *agr* group I *S. aureus* isolates, whatever their origin, were more prevalent for the presence of *seh*, *selq*, *selk* and/or *tst* virulence toxin genes and *sec* and/or *sell* virulence toxin genes (P<0.05%), respectively. Finally, it is also particularly noteworthy that, *selk*-positive and *selq*-positive isolates, were highly prevalent from pus/wound (P<0.05%).

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### References

1. Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339: 520-32.
2. McCormick JK, Yarwood JM, Schlievert PM (2001) Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol 55: 77-104.
3. Dinges MM, Orwin PM, Schlievert PM (2000) Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev 13: 16-34.
4. Bergdoll MS, Crass BA, Reiser RF, Robbins RN, Davis JP (1981) A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome *Staphylococcus aureus* isolates. Lancet 1: 1017-21.
5. Lina G, Bohach GA, Nair SP, Hiramatsu K, Jouvin-Marche E, et al. (2004) Standard nomenclature for the superantigens expressed by *Staphylococcus*. J Infect Dis 189: 2334-6.
6. Thomas D, Chou S, Dauwalder O, Lina G (2007) Diversity in *Staphylococcus aureus* enterotoxins. Chem Immunol Allergy 93: 24-41.
7. Ono HK, Omoe K, Imanishi K, Iwakabe Y, Hu DL, *et al.* (2008) Identification and characterization of two novel staphylococcal enterotoxins, types S and T. Infect Immun 76: 4999-5005.
8. McCormick JK, Tripp TJ, Llera AS, Sundberg EJ, Dinges MM, *et al.* (2003) Functional analysis of the TCR binding domain of toxic shock syndrome toxin-1 predicts further diversity in MHC class II/superantigen/TCR ternary complexes. J Immunol 171: 1385-92.
9. Marrack P, Kappler J (1990) The staphylococcal enterotoxins and their relatives. Science 248: 1066.
10. Holtfreter S, Broker BM (2005) Staphylococcal superantigens: do they play a role in sepsis? Arch Immunol Ther Exp (Warsz) 53:13-27.
11. Lindsay JA, Holden MT (2004) *Staphylococcus aureus*: superbug, super genome? Trends Microbiol 12: 378-85.

12. Schmidt H, Hensel M (2004) Pathogenicity islands in bacterial pathogenesis. Clin Microbiol Rev 17: 14-56.

13. Novick RP (2003) Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of *Staphylococcus aureus*. Plasmid 49: 93-105.

14. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, *et al.* (2002) Genome and virulence determinants of high virulence community-acquired MRSA. Lancet 359: 1819-27.

15. Novick RP, Ross HF, Projan SJ, Kornblum J, Kreiswirth B, et al. (1993) Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. Embo J 12: 3967-75.

16. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, et al. (2002) Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun 70: 631-41.

17. Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48: 1429-49.

18. Novick RP (2006) Staphylococcal pathogenesis and pathogenicity factors: genetics and regulation. In Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, and Rood JI (ed.), Gram-positive pathogens, 2nd ed. ASM Press, Washington, DC 26, pp 496-516.

19. Sambrook J, Fritsch EF, Maniatis T (1989) Extraction with phenol: chloroform. In Molecular Cloning: A Laboratory Manual, 2nd edn, Cold Spring Harbor Laboratory Press, Plainview, NY, pp E.3-E.4.;

20. Brakstad OG, Aasbakk K, Maeland JA (1992) Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J Clin Microbiol 30: 1654-60.

21. Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, *et al.* (1995) Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. J Clin Microbiol 33: 2864-7.

22. Holtfreter S, Grumann D, Schmudde M, Nguyen HT, Eichler P, *et al.* (2007) Clonal distribution of superantigen genes in clinical *Staphylococcus aureus* isolates. J Clin Microbiol 45:2669-80.

23. Gilot P, Lina G, Cochard T, Poutrel B (2002) Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of *Staphylococcus aureus* strains isolated from cows with mastitis. J Clin Microbiol 40: 4060-7.

24. Balaka B, Agbere A, Dagnra A, Baeta S, Kessie K, et al. (2005) Genital bacterial carriage during the last trimester of pregnancy and early-onset neonatal sepsis. Arch Pediatr 12:514-9.

25. Dykhuizen RS, Harvey G, Gould IM (1995) The high vaginal swab in general practice: clinical correlates of possible pathogens. Fam Pract 12: 155-8.

26. Muder RR, Brennen C, Rihs JD, Wagener MM, Obman A, et al., (2006) Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia. Clin Infect Dis 42: 46-50.

27. Sheth S, DiNubile MJ (1997) Clinical significance of *Staphylococcus aureus* bacteriuria without concurrent bacteremia. Clin Infect Dis 24: 1268-9.

28. Jensen AG, Wachmann CH, Espersen F, Scheibel J, Skinhoj P, et al. (2002) Treatment and outcome of *Staphylococcus aureus* bacteremia: a prospective study of 278 cases. Arch Intern Med 162:25-32.

29. Weems JJJr (2001) The many faces of *Staphylococcus aureus* infection. Recognizing and managing its life-threatening manifestations. Postgrad Med 110: 24-6, 29-31, 35-6.

30. Kesah C, Ben Redjeb S, Odugbemi TO, Boye CS, Dosso M, et al., (2003) Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. Clin Microbiol Infect 9:153-6.

31. Ben Nejma M, Mastouri M, Bel Hadj Jrad B, Nour M (2008) Characterization of ST80 Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* clone in Tunisia. Diagn Microbiol Infect Dis. Xxx-xxx.

32. Layer F, Ghebremedhin B, Konig W, Konig B (2006) Heterogeneity of methicillin-susceptible *Staphylococcus aureus* strains at a German University Hospital implicates the circulating-strain pool as a potential source of emerging methicillin-resistant *S. aureus* clones. J Clin Microbiol 44: 2179-85.

33. Hu DL, Omoe K, Inoue F, Kasai T, Yasujima M, *et al.,* (2008) Comparative prevalence of superantigenic toxin genes in meticillin-resistant and meticillin-susceptible *Staphylococcus aureus* isolates. J Med Microbiol 57: 1106-12.

34. Udo EE, Al-Mufti S, Albert MJ (2009) The prevalence of antimicrobial resistance and carriage of virulence genes in *Staphylococcus aureus* isolated from food handlers in Kuwait City restaurants. BMC Res Notes 2:108.

35. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, *et al.* (2001) Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet 357: 1225-40.

36. Letertre C, Perelle S, Dilasser F, Fach P (2003) Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*. J Appl Microbiol 95: 38-43.

37. Jarraud S, Peyrat MA, Lim A, Tristan A, Bes M, et al., (2001) *egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J Immunol 166: 669-77.

38. Chiang YC, Liao WW, Fan CM, Pai WY, Chiou CS, et al. (2008) PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. Int J Food Microbiol 121: 66-73.

39. Chini V, Dimitracopoulos G, Spiliopoulou I (2006) Occurrence of the enterotoxin gene cluster and the toxic shock syndrome toxin 1 gene among clinical isolates of methicillin-resistant *Staphylococcus aureus* is related to clonal type and *agr* group. J Clin Microbiol 44: 1881-3.

40. Hwang SY, Kim SH, Jang EJ, Kwon NH, Park YK, *et al.* (2007) Novel multiplex PCR for the detection of the *Staphylococcus aureus* superantigen and its application to raw meat isolates in Korea. Int J Food Microbiol 117: 99-105.

41. Omoe K, Hu DL, Takahashi-Omoe H, Nakane A, Shinagawa K (2005) Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. FEMS Microbiol Lett 246: 191-8.

42. Thomas DY, Jarraud S, Lemercier B, Cozon G, Echasserieau K, *et al.* (2006) Staphylococcal enterotoxin-like toxins U2 and V, two new staphylococcal superantigens arising from recombination within the enterotoxin gene cluster. Infect Immun 74: 4724-34.

43. Sila J, Sauer P, Kolar M (2009) 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 153: 215-8.

44. Becker K, Friedrich AW, Lubritz G, Weilert M, Peters G, et al. (2003) Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. J Clin Microbiol 41: 1434-9.

45. Lindsay JA, Ruzin A, Ross HF, Kurepina N, Novick RP (1998) The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol Microbiol 29: 527-43.

46. Ji G, Beavis R, Novick RP (1997) Bacterial interference caused by autoinducing peptide variants. Science 276: 2027-30.

47. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F (2006) Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 193: 1495-503.

48. Peck KR, Baek JY, Song JH, Ko KS (2009) Comparison of genotypes and enterotoxin genes between *Staphylococcus aureus* isolates from blood and nasal colonizers in a Korean hospital. J Korean Med Sci 24: 585-91.

49. El-Huneidi W, Bdour S, Mahasneh A (2006) Detection of enterotoxin genes *seg*, *seh*, *sei*, and *sej* and of a novel aroA genotype in Jordanian clinical isolates of *Staphylococcus aureus*. Diagn Microbiol Infect Dis 56: 127-32.

50. Proft T, Fraser JD (2003) Bacterial superantigens. Clin Exp Immunol 133:299-306.

51. Zhang S, Iandolo JJ, Stewart GC (1998) The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (*sej*). FEMS Microbiol Lett 168: 227-33.

52. Omoe K, Hu DL, Takahashi-Omoe H, Nakane A, Shinagawa K (2003) Identification and characterization of a new staphylococcal enterotoxin-related putative toxin encoded by two kinds of plasmids. Infect Immun 71: 6088-94.