Relationships between toxin gene content and genetic background in nasal carried isolates of *Staphylococcus aureus* from Asturias, Spain

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Abstract

Staphylococcus aureus recovered from nasal carriers, producers and non-producers (43 isolates each) of classical pyrogenic toxin superantigens (PTSAgs), were screened for 17 additional PTSAg-genes by PCR. Percentages of 88.4 and 65.1 were positive for some new enterotoxin-gene, and 76.7 and 55.8 for enterotoxin-gene-clusters (*egc*-like), respectively. The 86 isolates belonged to 17 toxin-genotypes (all *eta-*, *etb-*, *etd-*, *see-* and *sep-*negative), and generated 40 *Sma*I-genomic profiles that in a dendrogram of similarity ($S \geqslant 0.7$) clustered into nine lineages and 11 non-clustered branches. Correlations between classical PTSAgs and *Sma*I-lineages were established and *egc*-like groupings appeared dispersed in six lineages.
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1. Introduction

The primary habitats of *Staphylococcus aureus* in humans are the mucosae of the nasopharynx where the bacteria exist as a persistent or transient member of the normal microbiota without causing any symptoms. Human carriers are the major infection source of *S. aureus*, which can cause both nosocomial and community-acquired diseases, which range from simple abscesses to fatal sepsis, plus toxinoses. For this complex set of diseases, *S. aureus* produces and secretes 30 or more specific pathogenicity factors that interfere with host defences [1]. Some *S. aureus* strains are able to produce staphylococcal enterotoxins (SEs), toxic shock syndrome toxin (TSST-1), and exfoliatins (ETs) which are involved in distinct pathologies but share common biological activities such as pyrogenicity, immunosupression, and non-specific T-cell proliferation and are therefore referred to as pyrogenic toxin superantigens (PTSAgs). Besides the common features, some PTSAgs are able to cause other symptoms, but only the SEs have emetic activity and cause primarily acute gastrointestinal damage [1–3]. SEs were initially discovered in studies of *S. aureus* implicated in poisoning outbreaks, and they were classified into distinct serological types. In recent years, increasing data resulting from partial or complete genome sequence analysis have allowed the identification of several new SE types [4–9]. Genes encoding PTSAgs are located in different genetic elements including prophages (*sea*, *see*, *sep*, *eta*), plasmids (*seb*, *sec1*,

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sed, sej, ser and etb), and pathogenicity islands (tst, seb, sec, sek–seo, seq, and etd) [6–10]. An enterotoxin gene cluster (egc) encoding a putative nursery of superantigens, with five genes (seg, sei, sem, sen, seo) and two pseudogenes (ψent1 and ψent 2) has been identified [4] and located in the sequenced genome of methicillin-resistant strains as a part of a pathogenicity island [6,8]. An egc-derivative encoding SEG, SEI and SEN variants and the new SEU (seu is the result of a 15 bp insertion into ψent1) was described two years later [5]. With respect to the frequency of S. aureus producing PTSAgs or carrying their genes, only the classical and well-recognised serological types (SEA to SED and TSST-1) have been strongly screened in isolates from different geographical areas, causing or not causing disease   (http://www.ncbi.nlm.nih.gov/PubMed/medline). Recent PCR-based studies indicate that the newly described SE-genes could be widely distributed among S. aureus [4,7,11–15], but due to their recent description, the incidence of each gene and the PTSAg-genotypes in S. aureus of different origin still has to be clarified. The interest of this clarification is also supported by a recent report showing that the egc-encoded PTSAgs are neutralized by human sera much less efficiently than are the classical PTSAgs, and that both the amounts and the spectrum of secreted PTSAgs differ between S. aureus carriage and S. aureus infection [16].

In the present study, 10 S. aureus control strains and 86 isolates recovered from nasal samples of healthy people, previously identified as producers or non-producers (half of each) of some of five classical PTSAgs (SEA to SED and TSST-1) [12,17–19], were subject to different experiments with two major aims. First, to ascertain in each set the proportion of isolates containing other PTSAg genes (here labeled as new), to determine PTSAg-genotypes, and to assess the presence of egc and distinctive SaPIs. Second, to discriminate nasal isolates into genomic types, in order to establish the genetic relationship within PTSAg-positive isolates, and between these and PTSAg-negative isolates.

2. Materials and methods

2.1. S. aureus isolates

The 86 S. aureus isolates analyzed in this study have been collected from the nasal cavities of the young adults considered as healthy carriers living in the Principality of Asturias, Spain, over a six-year period (1997–2002). They were analyzed for the presence of five major serological toxin types (SEA to SED and TSST-1) by reversed passive latex agglutination using two commercial kits, TST-RPLA and SET-RPLA (Oxoid, Hampshire, England), and their genes by PCR [12,17–19]. Of these isolates, 43 (set A) had been identified as producers and the other 43 (set B) as non-producers of the cited classical PTSAgs and carriers of the corresponding genes. The 10 strains compiled in Table 1 were used as controls in the different experiments.

2.2. Isolation of genomic DNA and detection of PTSAg-genes

Genomic DNA isolation and detection of genes encoding PTSAgs, by conventional and multiplex-polymerase chain reaction (PCR) were achieved as previously reported [12,19]. In all PCR-assays each isolate was tested, at least twice, and positive and negative controls were always included. The primers for the

Table 1
Genetic features of the S. aureus control strains used in this work

Strain	Prototype	Pyrogenic toxin superantigens genes
CECT 976 (ATCC 13565)[a]	SEA/sea	sea, (sed, sej, ser)[b]
CECT 4459[a]	SEB/seb	sea, (seb, sek, seq)[c] (seg, sei, sem, sen, seo)[d]
CECT 4465 (ATCC 19095)[a]	SEC/sec	(sec, sel, sem)[e] (seg, sei, sem, sen, seo, seu)[f]
CECT 4466 (ATCC 23235)[a]	SED/sed	(sed, sej, ser)[b] (seg, sei, sem, sen, seo)[d]
CECT 59 (ATCC 9114)[a]	PTSAg-negative	(seg, sei, sem, sen, seo, seu)[f]
ATCC 27664	SEE/see	see
LMUO M81	seh	seh
CNM 3194/98[a]	TSST-1/tst	tst, (seg, sei, sem, sen, seo, seu)[f]
CNM 3/99	ETB 2/etb	etb, (seg, sei, sem, sen, seo, seu)[f]
NCTC 8325[a,g]	TSAGg-negative	

  [a] SmaI-profiles shown in Fig. 1. CECT (Colección Española de Cultivos Tipo), ATCC (American Type Culture Collection), NCTC (National Collection of Type Cultures), CNM (Centro Nacional de Microbiología), LMUO (Laboratorio de Microbiología Universidad de Oviedo).
  [b] Gene-grouping associated to plasmids [7].
  [c] Gene-grouping associated to SaPI3 [10].
  [d] Gene-grouping named egc [4].
  [e] Gene-grouping associated to SaPI4 [10].
  [f] A variant of egc including the seu gene [5].
  [g] PFGE control [24].

following genes, and the size of the amplicons (in bp) generated by them, have been previously described: *see*-512 and *sej*-648 [18]; *sei*-576 and *sen*-680 [15]; *seh*-494 and *seg*-683 [20]; *sek*-278 and *seq*-285 [21]; *seu*-141 [5], *sel*-359, *sem*-473, *seo*-722, *sep*-276 and *ser*-700 [22], and *eta*-741, *etb*-629 and *etd*-376 [23]. In multiplex-PCR different sets of primers were assayed with the control strains. On the basis of amplicon profile and reproducibility, six primer-sets were selected to test the 86 nasal isolates: set-1 (*eta*, *etb*, *etd*), set-2 (*see*, *sej*), set-3 (*seg*, *seh*, *sei*), set-4 (*sem*, *sen*, *seu*), set-5 (*sel*, *seo*, *sep*), and set-6 (*seq*, *ser*). The *sek*-primers did not generate amplicons in the different combinations tested; for this reason they were tested alone. In this and previous works [12,17–19,22] using PCR-procedures to detect virulence genes, we have observed a high frequency of false negatives and a lower rate of false positives. This difficulty was minimized using isolated DNA, instead of bacterial culture, as template and testing all isolates at least twice. When results did not conform in the two assays and/or the gene-grouping of an isolate did not match with the expected genetic element in which should be inserted, the isolate was tested again by conventional PCR. Statistical comparison between producer and non-producer isolates was performed using the "two-sample tests of proportion independents" (software: Stata 6.0. Stata Corporation, TX, USA). Differences between groups were considered statistically significant if P values were ⩽0.05.

2.3. Macrorestriction-pulsed field gel electrophoresis analysis

Whole DNA from each *S. aureus* isolate was analyzed by macrorestriction-PFGE performed with *Sma*I by means of the CHEF-DRIII SYS220/240 (Bio-Rad Laboratories, S.A., Madrid, Spain) basically using the consensus protocol [24]. *Sma*I banding profiles were analyzed visually, recording the presence or absence of each band, and those showing one or more mismatching bands were considered different. *Sma*I-profiles were initially grouped according to the toxins produced by the isolates generating them and numbered. The similarity between *Sma*I-profiles was traced in two ways: number of mismatching fragments and cluster analysis. For the latter, an unweighted pair method with arithmetic averages (UPGMA) was used together with Jaccard's similarity coefficient (*S*) in the software Program MVSP 3.1 (Multivariate Statistics Package for PCs, RockWare Inc[R]). Isolates generating *Sma*I-profiles that differ from six or less bands (*S* ⩾ 0.7) were considered genetically related. The discrimination index (DI) (i.e. the probability that two unrelated isolates obtained from the population would be placed into different *Sma*I-profiles) was calculated by using Simpson's index of diversity (MVSP 3.1, 15).

3. Results

3.1. PCR detection of new PTSAg-genes in S. aureus isolates positive or negative for classical PTSAgs

Control strains and the two sets of nasal isolates were tested for 17 new PTSAg genes by PCR. Results showed that six of the eight PTSAg prototype strains contained some additional PTSAg gene to the one for which they are prototype, and the CECT 59 strain initially considered PTSAg-negative was found to be positive for six SE-genes (Table 1). All the 86 nasal isolates were negative for *eta*, *etb*, *etd*, *see* and *sep*, and 20 (23.3%) of them were negative for the 17 new PTSAg-genes. In Table 2 results are compiled from comparing the frequency of the new SE-genes and PTSAg-genotypes in the two sets. In set A, 38 isolates (86.4%) were positive for new SE-genes and the remaining five isolates carried only one classical PTSAg-gene (*sea* in three isolates, or *tst* in two isolates); while in set B, 28 (65.1%) isolates were positive for new SE-genes (statistically significant difference, $P = 0.0053$)

Some PTSAg-genes always appeared associated (Tables 1 and 2), and a part of these associations could be related with specific genetic supports: (i) *seo–sem–sen–*

Table 2
Frequency of new PTSAg genes and PTSAg genotypes among *S. aureus* isolates collected from nasal samples of healthy humans

Gene (No.)	Genotype: gene groupings (No.)
Set A[a]	
seg (33)	G1: *tst* (2)
seh (5)	G2: *tst*, *sea*, *egc2*[c] (11)
sei (33)	G3: *tst*, *egc2*[c] (3)
sej (5)	G4: *tst*, *seh*, *egc2*[c] (3)
sek (3)	G5: *sea* (3)
sel (7)	G6: *sea*, *sec*, *seh* (2)
sem (33)	G7: *sea*, *egc1*[d] (1)
sen (33)	G8: *seb−sek−seq*[e] (3)
seo (33)	G9: *seb*, *egc1*[d] (3)
ser (5)	G10: *sec–sel* [f], *egc1*[d]
seu (17)	G11: *sec–sel* [f], *sed–sej–seg* [g], *egc1*[d] (3)
	G12: *sed–sej–ser* [g], *egc1*[d] (2)
Set B[b]	
seg (23)	G13: *egc1*[d] (21)
seh (1)	G14: *egc2*[c] (3)
sei (23)	G15: *egc1*[d], *seh* (1)
sem (23)	G16: *seo* (2)
sen (23)	G17: *seq* (1)
seo (25)	G0: none (15)
seq (1)	
seu (3)	

[a] Set A includes 43 isolates producers of classical PTSAgs.
[b] Set B includes 43 isolates non-producers of classical PTSAgs.
[c] *egc2*, includes the *seg*, *sei*, *sem*, *sen*, *seo* and *seu* genes [5].
[d] *egc1*, includes the *seg*, *sei*, *sem*, *sen* and *seo* genes [4].
[e] Gene-grouping associated to SaPI3 [10].
[f] Gene-grouping associated to SaPI4 [10].
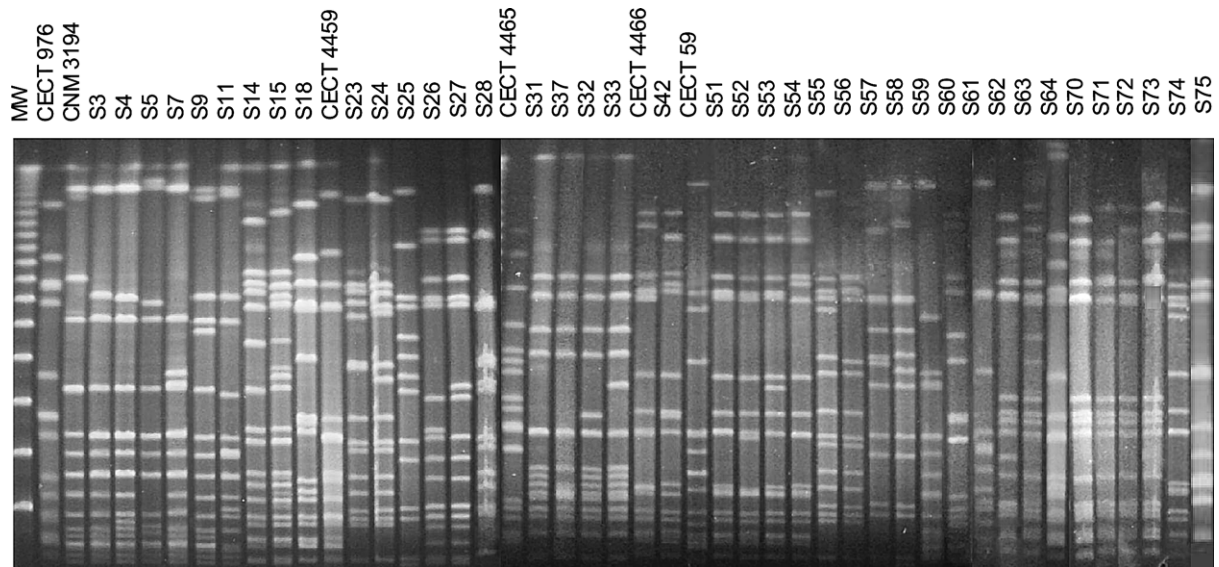[g] Gene-grouping associated to plasmids [7].

Fig. 1. *Sma*I-PFGE analysis of *S. aureus* isolates from nasal samples of healthy carriers and control strains. Lanes λ, lambda ladder PFGE Marker (New England Biolabs). Lane R, NCTC 8325 used as PFGE control; it lacks the 208-kb fragment present in the profile shown in [24]. Lanes 3–75, profiles generated by *S. aureus* isolates collected from human carriers. This is a composite figure, from three gels analysed under identical PFGE conditions. The distribution of isolates into profiles is shown in Table 2.

seg–sei with *egc* [4] or its variant also carrying *seu* [5]. Here, both were considered *egc*-like and labelled *egc1* and *egc2*, respectively. The first, *egc1*, was also present in SEB and SED prototype strains; and the second, *egc2*, in SEC, TSST-1 and ETB2 prototype strains as well as in CECT 59 (Table 1). (ii) *seb–sek–seq*, compatible with the presence of SaPI3 [8,10], was found in only three SEB nasal isolates and in the SEB-prototype; whereas *seb* (but not *sek–seq*) appeared in three other

SEB nasal isolates. (iii) *sec–sel–sem* (this last gene also forms part of *egc*), compatible with the presence of some SaPI4-like element [8,10] appeared in 10 nasal isolates and in the SEC prototype strain. The two remaining *sec*-positive isolates were *sel–sem*-negative, but were *sea*- and *seh*-positive. (iv) SEA- and SED-prototype and the eight *sed*-positive nasal isolates (and only these) were *sej*- and *ser*-positive. The *sed–sej–ser* grouping has been described as plasmid-located [7,18]. (v) These

Table 3
Relationships between PTSAg genotypes with *Sma*I genomic lineages and non-clustered profiles of *S. aureus* collected from nasal samples of healthy carriers

PTSAg-genotypes vs. *Sma*I-lineages/profiles[a]		*Sma*I-lineages/profiles[a] vs. PTSAg-genotypes	
Genotype (No.)	Lineage/profile[a] (No.)	Lineage/profile[a] (No.)	Genotype (No.)
G1 (2)	L1 (2)	L1 (19)	G1 (2), G2 (11), G3 (3) G4 (3)
G2 (11)	L1 (11)	L2 (4)	G5 (2), G6 (2)
G3 (3)	L1(3)	L3 (2)	G5, G7
G4 (3)	L1(3)	L4 (6)	G9 (2), G13 (4)
G5 (3)	L2 (2), L3	L5(2)	G8 (2)
G6 (2)	L2 (2)	L6 (15)	G10 (7), G11 (3), G13 (5)
G8 (3)	L5 (2), S28	L7 (12)	G12 (2), G13 (10)
G9 (2)	L4 (2)	L8 (2)	G13, G14
G10 (10)	L6 (7)	S59 (2)	G14 (2)
G11 (3)	L6 (3)	L9 (12)	G0 (12)
G12 (2)	L7 (2)	Others[b]	
G13 (21)	L4 (4), L6 (5), L7 (10), L8, S60		
G14 (3)	S57, S59 (2)		
G15	S61		
G16 (2)	S62, S63		
G0	L9 (12), S73 (3), S74, S75		

[a] Non-clustered profiles.
[b] With only one isolate. In brackets number of isolates ≥ 2.

gene-groupings appeared alone or combined with one another and/or with other genes forming the 17 PTSAg-genotypes recorded in Table 2.

3.2. PFGE-macrorestriction analysis of genomic DNA

Control strains and nasal isolates were also analyzed by PFGE-macrorestriction performed with *Sma*I. In order to define profiles only *Sma*I fragments between ≈25 and 800-kb were considered. Nasal isolates were differentiated into 40 *Sma*I-profiles, and the 10 control

strains generated 10 additional *Sma*I-profiles (in Fig. 1 only six of these are shown). The number of nasal isolates representing each *Sma*I-profile is compiled in Table 3. This procedure yielded DI = 0.96 for the 86 nasal isolates, and DI = 0.97 when the control strains were included (n = 96).

On the basis of the coefficient of similarity of the *Sma*I-profiles shown in Fig. 1, a dendrogram was constructed; and at a cut-off point of S = 0.7 nine clusters (considered as lineages, L1 to L9), and 11 non-clustered branches were revealed (Fig. 2). Lineages were categorized as major when they included between three and seven profiles, and minor if only two profiles were grouped. Relevant relationships between major *Sma*I-lineages and PTSAg-genotypes are the following. L1 was found to include organisms with four PTSAg genotypes (G1–G4) with the common feature of being *tst*-positive and, except G1, also being *egc2*-positive. L4, L6 and L7 group together isolates with the common feature of being *egc1*-positive, and a part of these isolates were also positive for some other lineage-associated PTSAg-gene or gene-grouping: L4 with *seb*; L6 with *sec–sel–sem* alone or together with *sed–sej–ser*; and L7 with *sed–sej–ser*. In the remaining major lineage, L9, only PTSAg-negative isolates (G0) were found.

4. Discussion

Findings generated from the present investigation provide us with some answers to the questions raised in relation with both epidemiological and genetic aspects of *S. aureus* carrying PTSAg-genes and colonizing the nasal mucosae of healthy people. With respect to the first aim (to ascertain the proportion of isolates containing new PTSAg-genes in two sets of isolates: containing and not containing classical PTSAg-genes), a high frequency of new PTSAg-genes was found, being higher (88.4%) in the first set than in the second set (65.1%), and in both sets the genes related with *egc*-like groupings were highly prevalent. The work reporting *egc* existence already indicated the high prevalence of *seg–sei* (and thus *egc*) in strains causing toxemia or suppurative infections as well as from nasal carriers [4]. Conversely, in the present study it was also found that none of the nasal isolates contained genes encoding exfoliatin toxins, nor SEE or SEP, and nearly one-quarter lacked all the 17 new PTSAg genes screened. In other previous works, some of the new SE-genes have also been screened and were found in higher frequencies than classical SE-genes in *S. aureus* from human carriers [7, 11–14]. Our experience supports these reported data, and also indicates that the real proportion of *S. aureus* containing PTSAg-genes could be two or three times higher than that reported when only the classical-PTSAgs are screened. It is noteworthy that in a major

Fig. 2. Dendrogram of similarity illustrating genetic relatedness of the *Sma*I macrorestriction fragment profiles generated by *S. aureus*. Cluster analysis was performed by the Jaccard similarity coefficient and the unweighted pair group method. At a cut-off point of S = 0.7 nine clusters considered as lineages (labelled L1– L9) and 14 branches were found. The PTSAg-genotypes of control strains and nasal isolates (G0 and G1–G17) are shown in Tables 1 and 2, respectively. The number of nasal isolates falling into each PTSAg-genotype, *Sma*I-lineage, and *Sma*I-branch are compiled in Tables 2 and 3.

paper of this field [14] it was examined the relationship between human disease and 33 putative virulence determinants (13 of them genes encoding PTSAgs), concluding that eight determinants (*fnbA*, *cna*, *sdrE*, *sea*, *sej*, *eta*, *hlg*, and *ica*) were significantly commoner in invasive than in carried isolates. Only three of these eight determinants (*eta*, *sea* and *sej*) are being screened in our laboratory [12,18,19] in isolates from healthy people. Comparing results on gene frequency and gene-groupings from the 174 isolates analyzed in [14] and the 150 isolates analyzed in our laboratory, the following facts could be noticeable: (i) in the Peacock et al. study, 11 (6%) isolates were *eta*-positive (25% of the invasive isolates), while all isolates tested by us were negative. (ii) Regarding the two enterotoxin genes: *sea* appeared in a similar frequency and mainly in association with *tst* in both studies; while *sej* appeared more frequently in carriage and invasive isolates (7% and 25% respectively, and frequently not associated with *sed*) in [14] than in our laboratory (3.6% in carriage and always *sed–ser* associated, [18]).

In *S. aureus* colonizing nasal mucosae the maintaining of several PTSAg-genes suggests benefits for the bacteria and for the host, but these benefits are not fully understood. In the first case, it has been hypothesized that the apparent redundancy of the superantigens encoded by *egc* confers a selective advantage towards colonization and/or invasion of humans [4,5,14,15]. In the second case, superantigens may play an important role in modulating the host immune response, being able to establish long-term anti-tumour activity and to enhance antibody production [2,3]. On the other hand are the negative effects of PTSAgs for the host, due to their toxic and superantigenic potential. These functions reside in two separate domains that probably have different targets. Theoretically, isolates in possession of multiple PTSA-genes should have a strong potential for causing toxinoses, together with a marked capacity for stimulating polyclonal T-cell proliferation and hence for inducing several deleterious effects in their host [1–4].

With respect to the genetic elements carrying PTSAg genes, in addition to the above observations the following are worthy of note. The sequencing of the partial or complete genome of *S. aureus* strains [6,8,10] has shown that some SaPIs contain distinctive PTSAg-genes or gene-groupings: (i) *egc1* forms part of SaPIn3/SaPIm3 included in the υSaβ family that harbours transposase genes, indicating that transposons may have been the origin of these islands [6,8], (ii) *tst* forms part of other islands (SaPI1, SaPI2, SaPIbov) included in the υSa1–4 family, which harbours integrase genes as putative elements of genetic mobility [6,8,10]. In some of these islands, *tst* is the single PTSAg-gene but in other islands *tst* is linked to *sek–seq* or *sec–sel*. In our study, none of the 19 *tst*-isolates (nor the TSST-1 control strain, CNM 3194/98) additionally contained some of the latter four

genes, suggesting that *tst* could form part of SaPI2 [10] or some other non-identified genetic element. (iii) *seb–sek–seq* forms part of SaPI3 [8,10] and in the present work it was found in three nasal isolates and in the SEB-prototype strain (CECT 4459). Three other nasal isolates carried *seb* but not *sek–seq*, suggesting that the *seb* genetic element is other than SaPI3. (iv) *sec–sel–sem* forms part of SaPI4 [8,10] and was present in this work in 10 nasal isolates and in the SEC-prototype strain (CECT 4465, ATCC 19095). The two other SEC-isolates contain *sec* in addition to *sea* and *seh* but not *sel–sem*, suggesting that the *sec* genetic element is other than SaPI4. These data indicated the detection of genes encoding PTSAgs by PCR as a useful tool for ascertaining the presence of both known and "new or not-described" genetic elements encoding PTSAgs. However, in order to confirm the existence of the "new" genetic elements suggested by the present work, additional investigation will be required.

Regarding the second aim (to ascertain the genetic relationship of nasal isolates carrying identical and different PTSAg-genotypes or PTSAg-lacking) a high heterogeneity of *Sma*I macrorestiction profiles was found. Some of these profiles could be clustered at different levels of similarity. Organisms generating *Sma*I-profiles with similarity ⩾70% were considered genetically related and members of the same lineage. Isolates carrying "new" SE-genes generated 31 out of the 40 *Sma*I-profiles and fell into eight of the nine *Sma*I-lineages. A relationship between *Sma*I-lineages and specific PTSAg-genes and PTSAg-genotypes was also revealed: L1 with genotypes containing *tst* (alone or together with *egc2*, or *egc2-sea*); L2 and L3 with genotypes containing *sea* (alone or together with other *se*-genes); L5 with genotypes containing *seb–sek–seq*; L4, L6, L7 and L8 with genotypes containing *egc1* alone or together with other genes (*seb* in L4; *sec–sel–sem* alone or together with *sed–sej–ser* in L6); and *sed–sej–ser* with L7. It is noteworthy that the plasmid location of *sed–sej–ser* has previously been confirmed by plasmid-hybridization assays in the five SED-nasal isolates and in the SEA- and SED-prototype strains, the plasmid size being between 33 and 36-kb [18]. Also of note is the broad dispersion of *egc*-like groupings among *Sma*I-profiles showing a high genetic heterogeneity and related with different PTSAg-genotypes. The discovery of SEU [5] in four out of 24 isolates selected as *seg–sei*-positive led these authors to suggest that further study of the prevalence of the *seu* gene in isolates of different origin would be useful [5]. Following this suggestion, some new information regarding two aspects of *seg2* is shown: first, its relatively high frequency in the total (23.2%) and within the *seg–sei*-positive (34.5%) isolates; and second, its presence in isolates also containing *tst* or *tst–sea* that form part of a distinctive *Sma*I-lineage (L1). On the other hand, using multilocus sequence typing, Peacock

et al. [14] grouped *S. aureus* into seven major clonal complexes that were related with nine virulence determinants (including *sea*, *sej* and *tst*). These authors found that most part of *sea* and *tst* isolates were clustered into a same clonal complex, whereas the *sej* isolates were distributed into this and other four clonal complexes. Our results from *Sma*I macrorestriction are concordant. In fact 11 out of the 17 *sea*-positive and all the 22 *tst*-positive isolates (11 of them positive for both genes) generated *Sma*I profiles with >70% of similarity, being considered as members of a same lineage which did not include *tst*-negative isolates. However, *sea* and *tst* were inserted in different *Sma*I-fragments, as revealed by hybridization [18], and were mediated by different genetic elements: prophages and SaPIs, respectively [6,8,10]. The wide dispersion of *sej* between clonal complexes [14,15] and *Sma*I-lineages [17, this work], could be in relation with its plasmid location.

Finally we wish to highlight that in previous works [12, unpublished data], we identified the *S. aureus* organisms implicated in four food poisoning outbreaks occurring in Asturias. Among the identified food-isolates some were SEA/*sea* or SEC/*sec–sel–egc1* or *egc1* and generated *Sma*I-profiles falling into L3, L6, and L7, respectively; moreover, and in the first case one food handler could be considered the infection source. It is also epidemiologically interesting that both SEA and SEC isolates generating similar *Sma*1-profiles to those falling into L3 and L5 have been implicated in food-poisoning outbreaks occurring in Japan [25] and Taiwan [26,27]. These findings suggest the pandemic character of both *S. aureus* lineages. In addition, it is noteworthy that *Sma*I-profiles of nasal isolates were distinct from those generated by isolates causing subclinical mastitis in dairy cows in Asturias, a dairy region, but in both human and bovine isolates the *egc*-like genes were the most frequent PTSAg-genes found [22].

Acknowledgements

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