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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.

Keywords: *Staphylococcus aureus*; Enterotoxins; Superantigens; PCR; PFGE

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].