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Population structure and exotoxin gene content of methicillin-susceptible *Staphylococcus aureus* from Spanish healthy carriers

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ABSTRACT

The population structure of 111 methicillin-susceptible *Staphylococcus aureus* (MSSA), recovered in Spain from healthy and risk-free carriers was investigated using pulsed-field gel electrophoresis (PFGE), *spa* (staphylococcal protein A) typing, multi locus sequence typing (MLST) and the accessory gene regulator (*agr*). Results from the different techniques were highly concordant, and revealed twelve clonal complexes (CCs): CC30 (27%), CC5 (18.9%), CC45 (16.2%), CC15 (11.7%), CC25 (8.1%), CC1, CC9 (3.6% each), CC59, CC97 and CC121 (2.7% each), CC72 (1.8%) and CC8 (0.9%). Isolates with genetic backgrounds of hospital-acquired MSSA were detected and, consistent with the ability of diverse MSSA to act as recipients of the SCC*mec* cassette, a MSSA isolate from a healthy carrier shared the ST, *spa*-type and *agr*-type of a MRSA clone recovered in a hospital of the same region. All except two fragments of the PGFE-profiles of these isolates were identical, and the differential fragment of the MRSA carried *mecA*. Analyses of the exotoxin gene content of the nasal isolates revealed an increase in the number of exotoxin genes over time. This, together with the detection of *lukPV* and the high frequency of *tst*, exfoliatin and enterotoxin genes, is worrisome and requires further surveillance.

1. Introduction

Staphylococcus aureus is an important human pathogen capable of causing a wide variety of diseases, ranging from mild skin infections and food poisoning to life-threatening conditions, such as deep abscesses, osteomyelitis, pneumonia, infective endocarditis and sepsis [1]. Despite this, *S. aureus* is primarily a member of the natural microbiota of the skin and mucosa of human beings, with estimations of about 20%–30% for persistent carriage [2,3]. The anterior nares are considered as the main ecological niche of *S. aureus*, and nasal carriage is a risk factor for development of infection, through autoinoculation with the own resident strain [4,5]. The ability of *S. aureus* to cause disease depends on virulence factors, many encoded by genes carried on mobile genetic elements (MGEs) like genomic islands (νSaβ), pathogenicity islands (SaPIs), bacteriophages and plasmids [6,7]. Disease may result from the action of a single virulence factor, as in some toxin-mediated diseases, or may be due to the cumulative effects of several factors [1,8].

To understand the relationship between nasal carriage and subsequent disease, insights into the genetic diversity and population structure of *S. aureus* collections derived from healthy carriers are essential [2]. However, in comparison with *S. aureus* of clinical origin, the information on *S. aureus* from healthy carriers is relatively limited. In the present study, nasal carriage isolates from Spanish healthy students with no known association with health-care facilities were subjected to molecular typing based on pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), type of accessory gene regulatory (*agr*) locus, and exotoxin gene content. The obtained results were compared with data available for other *S. aureus* collections, both from healthy carriers and health-care facilities including two hospitals located in the same Spanish region, the Central University Hospital of Asturias (HUCA) and the Monte Naranco Hospital (MNH) [9–11].

2. Methods

2.1. Strain collection

A total of 111 isolates, conserved at the collection of the Laboratory of Microbiology of the University of Oviedo (LMUO), were included in this study. The isolates were collected as part of

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laboratory training, where nasal swabs were taken from biology students attending courses on Health Microbiology taught at the University of Oviedo (Spain) over several years. All individuals were properly informed of the study, and the sampling procedure does not represent a health risk. The swabs were plated on Mannitol Salt Agar (Oxoid, Madrid, Spain) and incubated for 36–48 h. Single, well-isolated colonies with the typical appearance of *S. aureus* were subcultured, and identification was confirmed biochemically by API Staph system (bioMerieux, Marcy-l'Etolie, France), DNase production (DNase test agar; Oxoid) and coagulase production (Slidex Staph Plus; bioMerieux). None of the isolates was MRSA and all tested negative for the *mecA* gene by PCR amplification (not shown). Sixty eight out of the 111 isolates, from the 1997 to 2002 period, have been previously typed by SmaI-PFGE and screened for exotoxin gene content [12], but were further characterised in the present study.

2.2. Macrorestriction-PFGE analysis

Forty three additional isolates from 2004 to 2006 were subjected to macrorestriction-PFGE. After SmaI digestion, PFGE was performed using the CHEF-DRIII SYS220/240 system (Bio-Rad Laboratories, S.A., Madrid, Spain), according to Murchan et al. [13]. Visual assignment of the PFGE type was performed on the basis of the overall band-pattern. Relationships between profiles were established by the unweighted pair method with arithmetic averages (UPGMA), using the Jaccard coefficient of similarity (*J*) in the software Program MVSP version 3.1 (Multivariate Statistics Package for PCs; RockWare Inc). *S. aureus* NCTC 8325 (National Collection of Type Cultures, United Kingdom) was included as control strain for PFGE analysis. Selected PFGE profiles were transferred onto membranes and hybridized with a *mecA* probe labelled by the commercial procedure PCR DIG Labelling Mix (Roche Diagnostics, Spain).

2.3. Sequence-based typing methods

The 111 isolates from healthy carriers were subjected to *spa*-typing [14], and 20 of them, including one or two representatives from each SmaI-PFGE cluster, and showing different *spa*-types, were also analysed by MLST [15]. Genomic DNA was purified by the phenol–chloroform and proteinase K (50 μg/ml; Roche Diagnostics, Spain) method [16], preceded by a lysis step with lysostaphin (0.02 μg/ml, Sigma, St. Louis, USA). The DNA was then used as template in PCR amplifications of the *spa* (*spa*-typing), *arcC, aroE, gplF, gmk, pta, tpi* and *yqiL* (MLST) genes. PCR products generated by both techniques were purified with the GFX PCR DNA Gel Band Purification kit (GE Healthcare, Madrid, Spain), and sequenced at Secugen S.L (Madrid, Spain) or Macrogen Inc (Seoul, Korea). The Ridom Spa Server (http://spaserver.ridom.de/) and the MLST website (http://www.mlst.net) were used to assign *spa* types and sequence types (ST), respectively. Isolates were grouped into a single CC when at least five of the seven housekeeping genes included in the MLST scheme were identical [15].

2.4. Virulence gene content and agr typing

The 43 isolates from the 2004 to 2006 period were also tested by simplex PCR for virulence genes encoding haemolysins (*hla, hlb, hld, hlg, hlg*-variant), leukotoxins (*lukED, lukM, lukPV*), exfoliatins (*eta, etb, etd*), toxic shock syndrome toxin (*tst*), enterotoxins (*sea, seb, sec, sed, see, seg, seh, sei, ser, ses, set*), and enterotoxin-like toxins (*selj, selk, sell, selm, seln, selo, selp, selq, selu*); as well as for markers of νSaβ genomic islands (*splF, bsaB*) and SaPIs (*ear*) using primers previously reported [17]. In addition, the 111 isolates were tested by simplex PCR amplifications for the type of *agr* locus (*agrI, agrII, agrIII, agrIV*), as reported [17]. A dendogram of similarity showing the clustering of the isolates according to virulence gene profiles was constructed by using the Jaccard coefficient of similarity (BioNumerics version 6.6., Applied Maths).

2.5. Statistical methods

The discrimination index (DI; the probability that two unrelated isolates would be assigned to different types or profiles) was calculated using Simpson's index of diversity [18]. Statistical comparisons were performed using the χ^2 test. Differences between groups were considered statistically significant if *P* values were <0.05.

3. Results

3.1. Genotypic typing of the isolates

In all, 61 distinct PFGE profiles were recognized among the 111 isolates, yielding a DI of 0.97. The 43 isolates recovered in Asturias from young healthy carriers during 2004–2006 generated 33 SmaI-PFGE profiles (Fig. 1). Eight of them (S3, S11, S31, S33, S51, S52, S55,

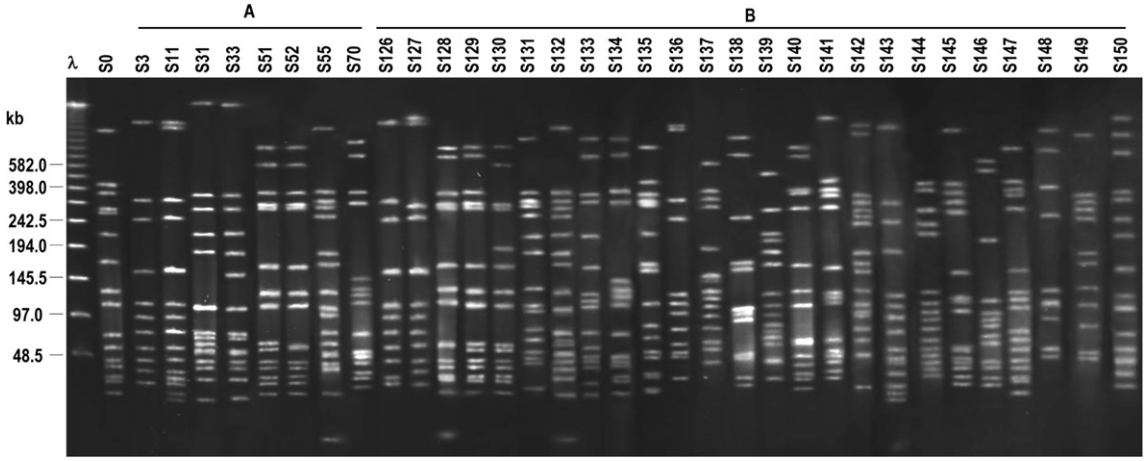

Fig. 1. SmaI-PFGE analysis of *S. aureus* isolates recovered between 2004 and 2006 from healthy nasal carriers. A, profiles also found in 1997–2001; B, new profiles detected in 2004–2006. Lane λ, lambda ladder PFG Marker (New England Biolabs); lanes S, SmaI profiles, following and continuing the nomenclature used by Fueyo et al. [12,19,20]. S0, SmaI profile of strain NCTC 8325 used as control.