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Original article

Enterotoxins and toxic shock syndrome toxin in *Staphylococcus aureus* recovered from human nasal carriers and manually handled foods: epidemiological and genetic findings

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Abstract

A set of 269 *Staphylococcus aureus* isolates recovered from nasal carriers and manually handled foods in a region of Spain was analyzed for pyrogenic toxin production and toxin genes. Fifty-seven isolates producing at least one of four enterotoxins (SEA, SEB, SEC, SED), 10 isolates producing only toxic shock syndrome toxin (TSST-1), and 10 isolates producing both toxin types were found. The 77 toxigenic isolates were discriminated into 36 *Sma*I genomic and 13 *Eco*RI plasmid profiles. A strong relationship between toxin profiles with both *Sma*I genomic and *Eco*RI plasmid profiles was revealed. *Sma*I genomic profiles showing six or less mismatching fragments and similarity coefficient ≥0.7 were included in a lineage. Eight lineages were differentiated; six of them grouped both human and food isolates and two of these also included outbreak-implicated isolates. Two lineages, represented by TSST-SEA and TSST-1, on the one hand, and SEC and SEC-SED isolates, on the other hand, were the most frequent, but only the second was outbreak-related. When *Sma*I genomic and *Eco*RI plasmid profiles were hybridized with *tst*, *sea*, *seb*, and *sec* toxin probes, it was observed that each probe mapped on a different *Sma*I fragment from isolates falling into the same lineage. All of the probes only mapped on genomic fragments, but *sed* also mapped on three plasmid fragments. When *sej* and *ser* probes were included, they mapped together with *sed* on the chromosome and on the plasmids. Two plasmids (ca. 33 and 36 kb) carried the expected *sed-sej-ser* genes, while the other (ca. 53.5 kb) carried *sed-sej* and *ser*-like genes. The latter plasmid and the chromosomal location of *sed-sej-ser* are new findings from this study.

Keywords: *Staphylococcus aureus*; Enterotoxins; TSST-1; PFGE; Plasmids; Toxin genes

1. Introduction

Staphylococcus aureus causes a wide variety of infections, from simple abscesses to fatal sepsis, plus toxinoses such as food poisoning and toxic shock syndrome. The symptoms of food poisoning in humans are mainly due to the secretion of emetic and pyrogenic toxins named staphylococcal enterotoxins (SEs). These toxins are single-chain proteins, and today, at least those designated SEA to SEE, SEG to SER, and SEU have been identified [1–7]. A further staphylococcal exotoxin involved in toxic shock syndrome toxin (TSST-1) was initially designated SEF [8] but does not have the "in vivo" biological activities of true SEs. Both SEs and TSST-1 are superantigens, which have the ability to stimulate large populations of T cells with a particular Vβ element of the T-cell receptor [1–3,7,9,10]. These toxins are encoded by genes associated with mobile (and therefore variable) genetic elements, such as prophages, *S. aureus* pathogenicity islands (SaPIs), and plasmids [2–7,11,12]. In humans, the primary habitat of *S. aureus* is the mucous membrane of the nasophar-

Abbreviations: DI, discrimination index; E1 to E13, *Eco*RI profiles numbers 1–13; L1 to L8, *Sma*I lineages numbers 1–8; LMUO, Laboratorio de Microbiología de la Universidad de Oviedo; PA, Principality of Asturias; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; PRPs, plasmid restriction profiles; pUO-SED, plasmid of University of Oviedo-Staphylococci Enterotoxin D; *S*1 to *S*36, *Sma*I profiles numbers 1–36; SaPIs, *Staphylococcus aureus* pathogenicity islands; SEA, SEB, SEC and SED, staphylococcal enterotoxins A, B, C, and D, respectively; TSST-1, toxic shock syndrome toxin.

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ynx, where it exists as a persistent or transient member of the normal microbiota without causing any symptoms. Organisms present in the nose may contaminate the hands; nasal carriers can thus become skin carriers, and these microorganisms can therefore be easily transmitted into manually handled foods. When colonizing food, some *S. aureus* excrete SEs that can cause poisoning after the ingestion of contaminated food. It also applies to cooked food due to the thermoresistance of the SEs [1,2,4,13].

This study was performed in a set of *S. aureus* isolates recovered from nasal carriers and manually handled foods in the Principality of Asturias (PA), Spain, in order to answer questions such as (i) what is the proportion of isolates producing the main serological types of toxins, SEA to SED and TSST-1, or carrying their genes? (ii) are the toxigenic isolates genetically homogeneous or heterogeneous? (iii) does a correlation exist between toxin profiles and genetic types traced by macrorestriction genomic analysis? (iv) do toxin genes located in the chromosome and/or in plasmids conform with the reported data? (v) could the obtained information be useful for epidemiological and genetic purposes?.

2. Materials and methods

2.1. Isolates and sources of isolation

The 269 isolates analyzed in this study were collected from the nasal cavities of healthy carriers (138 isolates, one from each carrier) and manually handled foods (131 isolates), including dairy products (cheeses, creams and ice creams), cakes, raw meats, and stuffed foods (catering, and cooked foods suspected of poisoning) (Table 1). All nasal isolates were randomly selected from the isolate collection conserved in the University of Oviedo's Microbiology Laboratory (LMUO). They were collected from healthy adults, mainly students from different courses and disciplines, over the period 1996–2002. Food isolates were collected at this and other food microbiology laboratories from the PA during the same time period. Only seven of the food isolates were collected in outbreak investigations [14], five of them being SE-positives (Table 1). An epidemiological relationship between human and food isolates included in this study was not identified. The isolates recovered between 1996 and 1999 were tested for SEs by immunoassay and by conventional and multiplex polymerase chain reaction (PCR) procedures in previous works [15,16]. In both toxin production tests and genetic assays, the following *S. aureus* strains were used as controls: CECT (Colección Española de Cultivos Tipo) 976 SEA-prototype (ATCC 13565), 4459 SEB-prototype, 4465 SEC-prototype (ATCC 19095), 4466 SED-prototype (ATCC 23235), 59 SE-negative (ATCC 9144), and LMUO TSST-prototype. CECT 4466 was also used in plasmid analysis [5,12], and NCTC 8325 in pulsed-field gel electrophoresis (PFGE) analysis [17]. Statistical analysis between human and food isolates was performed using the two-sample tests of 'independent proportions' (software: Stata 6.0., Stata Corporation, TX, USA). Differences between groups were considered statistically significant if P values were ≤0.05.

2.2. Detection of SEs and TSST-1 by immunoassay and by PCR

The production of staphylococcal enterotoxins A, B, C, D and TSST-1 was determined by reversed passive latex agglutination using two commercial kits, SET-RPLA and TST-RPLA (Oxoid, Hampshire, UK), according to the manufacturer's recommendations. The presence of *sea*, *seb*, *sec*, *sed*, *sej*, *ser* and *tst* genes was screened by PCR. The primers for the first five genes were reported in [16]. Genes were designed for the *tst* (*tst*-1: AGCATCTACAAACGATAATATAAAGG and *tst*-2: CATTGTTATTTTC CAATAACCACCCG) and *ser* (*ser*-1: AAACCAGATCCAAGGCCTGGAG and *ser*-2: TCACATTGTAGTCAGGTGAACTT). The last two primer pairs were designed from the sequences reported in GenBank (accession number AP003135) and [5], and they generated amplicons of 481 and 700 bp, respectively. The PCR conditions have been described previously [16].

2.3. PFGE-macrorestriction and plasmid analysis

Whole DNA from each *S. aureus* isolate was analyzed by PFGE-macrorestriction performed with *Sma*I by means of the CHEF-DRIII SYS220/240 (Bio-Rad Laboratories, S.A., Madrid, Spain) using the recommended protocol [17]. In the first step, *Sma*I banding profiles (*Sma*I profiles) were visually analyzed, and their presence or absence was recorded for each band scored. In a second step, DNAs from isolates generating similar *Sma*I profiles were run together in the same gel. *Sma*I profiles showing one or more mismatching fragments were considered different, and those with ≤6 or less mismatching fragments were included in a visual grouping [17,18]. 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 [19]. Initially, the *Sma*I profiles were numbered according to the date of isolation of the first strain generating the profile, but for this paper they were grouped and relabeled according to the toxin or toxins produced (Fig. 1). In a third step, the similarity between the *Sma*I profiles shown in Fig. 1 was analyzed according to the 'unweighted pair method with arithmetic averages' (UPGMA) and Dice's similarity coefficient (S) (software: SPSS for Windows 10.01.SPSS Inc., 1999). The S values clustering *Sma*I profiles into lineages are indicated in Fig. 1.

Plasmid content was determined by the alkaline lysis method with lysostaphin (Sigma, St. Louis, USA) [20], followed or not by digestion with *Eco*RI and *Hin*dIII (Takara, Shuzo Co., Ltd., Japan) according to the manufacturer's protocol. In both cases, after electrophoresis, the agarose gels were stained with ethidium bromide and visualized by UV