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Molecular characterization of *Staphylococcus aureus* carrying the panton-valentine leucocidin genes in northern Spain

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Summary *Objectives:* To study the prevalence of the Panton-Valentine leucocidin (PVL) gene in methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* obtained in Gipuzkoa, northeastern area of the Basque Country, north-central Spain, and perform the molecular characterization of PVL-positive isolates.
Methods: Molecular studies comprised: PVL gene detection by PCR, staphylococcal chromosome cassette *mec* (SCC*mec*) typing, *spa* sequencing, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and detection of the arginine catabolic mobile element (ACME).
Results: Between 1978 and 2006, only two (0.3%) of the 686 MRSA isolates studied were positive for the PVL gene. This percentage increased between 2007 and 2009, when the PVL gene was detected in 30 of the 679 MRSA (4.4%) and in nine of the 1227 MSSA (0.7%) isolates.

The 41 PVL-positive isolates characterized had eight different sequence types (STs). Twenty-three MRSA PVL-positive isolates were ST8, *spa* type t008, seven of which were ACME positive, erythromycin-resistant and showed the PFGE pattern (90—100% similarity) of the USA300 clone. ST8 was also the most prevalent ST among the nine MSSA PVL-positive isolates.
Conclusion: The current epidemiology of PVL-positive MRSA in our region more closely resembles that of the USA rather than that of other European countries, being USA300 or USA300-like isolates the most prevalent ones.

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Introduction

Staphylococcus aureus is one of the major human pathogens being responsible for infections ranging from asymptomatic nasal and skin carriage to life-threatening diseases, such as endocarditis, bacteraemia and necrotizing pneumonia. Among the various virulence factors produced by *S. aureus* is the Panton-Valentine leukocidin (PVL), a two-component cytotoxin encoded by two contiguous genes, namely *lukS-PV* and *lukF-PV*, which reside in the genome of temperate bacteriophages.[1] Clinically, PVL-producing *S. aureus* strains have been associated with necrotic lesions such as furunculosis and severe necrotizing pneumonia.[2]

The PVL is produced by both methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA), but the epidemiology of PVL-positive *S. aureus* has been mainly linked to MRSA, especially to community-acquired MRSA (CA-MRSA). Molecularly, CA-MRSA isolates are characterized by the presence of the staphylococcal chromosome cassette *mec* (SCC*mec*) type IV and the *lukS-PV* and *lukF-PV* genes, which are considered molecular markers of the worldwide distribution of CA-MRSA, although not all CA-MRSA carry the PVL genes and SC*Cmec* IV.[3]

The current prevalence of the infections caused by PVL-positive CA-MRSA isolates vary between less than 5% in some European areas to more than 50% in some regions of the USA.[4–6] Most studies have revealed a clonal population structure of PVL-positive CA-MRSA, but the clonal distribution in Europe seems to differ from that described in the USA.[7,8] The most prevalent PVL-MRSA clones described so far by their sequence type (ST) are the ST80 in Europe, the ST8-PFGE type USA300 in the USA, and the ubiquous ST30 (Southwest Pacific clone).[3,9,10] A characteristic feature of the genome of the USA300 clone is the presence of the arginine catabolic mobile element (ACME), related to its capacity of growth and survival, an element probably transferred from *Staphylococcus epidermidis*.[11]

The aim of this work was to characterize the clinical picture, prevalence and molecular characteristics of PVL-positive MRSA and MSSA causing clinical infections in a region of northern Spain. The association between ST8-SCC*mec* IV PVL-positive isolates and the USA300 clone was also investigated.

Materials and methods

Isolates

All *S. aureus* included in this study were isolated from clinical samples received at the Microbiology Department of Hospital Donostia located in the city of Donostia-San Sebastian, province of Gipuzkoa, which covers an estimated population of 350,000 inhabitants. Gipuzkoa is located in the northeastern area of the Basque Country in north-central Spain, bordered by the Cantabric Sea and France to the north.

Due to the repeated isolation of *S. aureus* from many patients, especially in those with skin (wound and ulcer) infections, only one isolate per episode was considered for the study. No isolates from nasal or skin carriers were included. The clinical and travel history data of the two patients with a PVL-positive MRSA infection that occurred in 1997 and 2003, a local fisherman and an elderly woman with recurrent pretibial ulcers, were obtained by a retrospective personal interview with the physician attending these patients. The country of residence and recent travel history of the patients with a PVL-positive *S. aureus* infection since 2007 were prospectively requested to the physician in charge of each patient. *S. aureus* isolates were identified by colony morphology, Gram-staining, characteristic yellow colonies on mannitol-salt-agar and a positive result in catalase and coagulase tests.

The prevalence of the PVL genes was retrospectively investigated in 686/1369 (50.1%) of the MRSA isolates that had been randomly selected (one in two) and frozen for future studies between 1978 and 2006. From January 2007 to December 2009, the presence of the PVL genes was studied prospectively in 679/938 (72.4%) MRSA isolates, that corresponded to all non-duplicated MRSA isolates collected, excluding nasal and skin carriers. Of the 1365 MRSA isolates tested for the presence of the PVL gene, 773 were isolated from SSTI (56.6%), including 44 abscesses; 209 from respiratory samples (15.3%), 102 from blood cultures (7.5%), 75 from urine (5.5%), 33 from otic exudates (2.4%) and the remaining 173 from different origins (12.7%).

The prevalence of PVL genes was also prospectively investigated in one out of five MSSA isolates randomly selected and isolated between 2007 and 2009.Overall, 1233 (20.5%) of the 6016 MSSA isolates collected were studied. Of the 1232 MSSA isolates tested for the presence of the PVL gene, 1000 were isolated from SSTI (81.2%) including 12 isolates from abscesses, 123 from otic exudates (10%), 24 from blood cultures (1.9%), 4 from respiratory samples (0.3%) and 81 from other sites (6.6%).

Antimicrobial susceptibility

Antimicrobial susceptibility testing of all *S. aureus* isolates was performed by the disk-diffusion and/or the broth microdilution methods, according to the Clinical and Laboratory Standards Institute guidelines.[12] Methicillin susceptibility was established according to oxacillin and/or cefoxitin disk inhibition zones. In addition, all isolates were tested against penicillin, ampicillin, amoxicillin-clavulanate, cefazolin, erythromycin, clindamycin, ciprofloxacin, levofloxacin, gentamicin, tetracycline, rifampicin, vancomycin, teicoplanin, trimethoprim-sulfamethoxazole, linezolid, mupirocin and fusidic acid. *S. aureus* ATCC 29213 was used as a control.

Molecular techniques

Bacterial DNA was extracted using the easyMAG automated nucleic acid-extraction system (bioMérieux, Boxtel, The Netherlands) and the nucleic acid isolation kit according to the manufacturer's instructions. All isolates were screened for the presence of the PVL genes using previously described primers and conditions.[13]

Multilocus sequence typing (MLST) was performed on PVL-positive isolates as described in the MLST web site (www.mlst.net). PFGE genotyping was performed after