Molecular Epidemiology of Panton-Valentine Leukocidin-Positive *Staphylococcus aureus* in Spain: Emergence of the USA300 Clone in an Autochthonous Population[▽]

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We characterized all of the Panton-Valentine leukocidin (PVL)-positive *Staphylococcus aureus* isolates collected between 2005 and 2008 in the Bilbao, Spain, area. For the first time, the USA300 clone is reported as predominant among PVL-positive clones in a European autochthonous population, requiring active monitoring of the incidence of USA300 in Spain and throughout Europe.

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains harboring the Panton-Valentine leukocidin (PVL) gene have recently emerged worldwide. They correspond to new clones unrelated to the former hospital-acquired MRSA (HA-MRSA) clones (15, 27, 34). The epidemiology of these CA-MRSA strains differs from the United States to Europe, the USA300 clone (ST8-MRSA-IV-PVL⁺) and the European ST80 clone (ST80-MRSA-IV-PVL⁺) being predominant in their respective geographical areas. Nevertheless, previous studies indicated an atypical situation in Spain. ST8-MRSA-IV-PVL⁺ isolates, which are likely related to USA300 (but do not yet have a definitive molecular characterization and description), have been detected in Madrid and Barcelona (4, 5). The majority of the patients were immigrants from Latin American countries, suggesting imported cases. In this context, the aims of this study were to determine the prevalence of PVL-positive *S. aureus* isolates in the Bilbao area and characterize the circulating clones.

A total of 1,334 nonrepeated *S. aureus* isolates were collected from January 2005 to November 2008 at Basurto Hospital (700 beds, population of 355,000 inhabitants). Forty PVL-positive isolates were identified using real-time PCR screening (2). These isolates were extensively characterized using *agr* typing (18), *mecA* PCR, SCC*mec* typing and subtyping (19, 26), *spa* typing (16), multilocus sequence typing (14), pulsed-field gel electrophoresis (PFGE) (1, 25), antimicrobial drug resistance (6, 7), and virulence gene profiles (8). The detection of genes described as USA300 markers, including *arcA* (an indicator of the arginine catabolic mobile element [ACME]) and the signature AT repeat sequence, was performed by PCR (3,

10) using the USA11358 and USA300-0114 strains (kindly provided by B. Kreiswirth and F. DeLeo, respectively) as reference strains for the USA300 clone.

The prevalence of PVL was 3% (40/1334), 2.3% (23/983), and 4.8% (17/351) among *S. aureus*, MRSA, and methicillin-susceptible *S. aureus* (MSSA) isolates, respectively. The 40 PVL-positive isolates, mainly involved in skin and soft-tissue infections (SSTIs) ($n = 34$, 85%), were all community acquired (collected within 48 h after admission). Thirty patients (75%) were Spanish, one was Ecuadorian, one was Bolivian, and one was Ukrainian. No information about the nationalities of the other seven patients could be obtained, but their health identification numbers corresponded to those of foreign citizens. Put together, these results suggest that the isolates collected in the hospital likely represented only the tip of the iceberg in Spain and that the issue of PVL-positive *S. aureus* in the community is widely underestimated in Spain.

Genetic characterization of the strains showed three major clonal clusters (based on *spa*-CC) grouping 85% of the isolates. The genetic markers, clinical data, and antimicrobial profiles are summarized in Tables 1 and 2. Twenty-four isolates (20 MRSA and 4 MSSA) were considered to belong to the USA300 clone or to be highly related. They shared the following characteristics: ST8, *agr*-I, and *spa*-CC008 (*spa*-t008 or related). PFGE revealed three highly related patterns, A, B, and C. The seven isolates with PFGE pattern A harbored features (*mecA*, SCC*mec* IVa, *arcA*, *sek* and *seq*, and the AT repeat signature [except *spa*-t068 isolates, $n = 2$]) matching those of the USA300 clone (reference strains). All isolates were resistant to beta-lactams, erythromycin, kanamycin, and fluoroquinolones. On the basis of these characteristics, these isolates were considered to belong strictly to the epidemic strain of the USA300 clone as defined by the USA300-0114 PFGE pattern (32). PFGE patterns B and C were highly related to USA300 pattern A (homology, >75.6%). Pattern B clustered 16 isolates (12 MRSA and 4 MSSA) that harbored the AT repeat signature, but the *arcA* gene was not detected. The antimicrobial resistance profiles were highly variable, but

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TABLE 1. Characteristics of 40 PVL-positive isolates from Basurto Hospital

<i>spa</i> -CC (no. of isolates)	<i>agr</i> allele	PFGE pattern ^a	No. ^b of patients	<i>spa</i> type	Repeats	ST (no. of isolates tested)	<i>mecA</i> gene	SCC <i>mec</i>	≥6 AT repeats	<i>arcA</i>	Toxin genes
008 (24)	1	A	5 (1)	t008	11-19-12-21-17-34-24-34-22-25	8 (4)	+	IVa	+	+	<i>selk, selq</i>
		A	2 (1)	t068	11-19-19-12-21-17-34-24-34-22-25	8 (2)	+	IVa	—	+	<i>selk, selq</i>
		B	3	t008	11-19-12-21-17-34-24-34-22-25	8 (1)	+	IVc	+	—	<i>selk, selq</i>
		B	2 (2)	t008	11-19-12-21-17-34-24-34-22-25	8 (1)	—	—	+	—	<i>selk, selq</i>
		B	8	t008	11-19-12-21-17-34-24-34-22-25	8 (2)	+	IVc	+	—	All negative
		B	1 (1)	t1635	11-19-12-21-17-34-25	8 (1)	—	—	+	—	<i>sed, selk, selq, selr</i>
		B	1 (1)	t3060	11-19-12-21-17-34-24-34-17-22-25	8 (1)	+	IVc	+	—	<i>selk, selq</i>
		B	1	t4882	11-19-19-12-21-17-34-25	8 (1)	—	—	+	—	<i>sed, selk, selq, selr</i>
		C	1	t1635	11-19-12-21-17-34-25	8 (1)	+	IVa	+	—	<i>sed, selk, selq, selr</i>
433 (6)	3	ND ^c	1	t012	15-12-16-02-16-02-25-17-24-24	30 (1)	—	—	—	—	<i>sea, sem, seo, tst</i>
			2	t019	08-16-02-16-02-25-17-24	30 (1)	+	IVc	—	—	<i>sem, seo</i>
			2	t433	15-12-16-16-02-16-02-25-17	30 (1)	—	—	—	—	<i>sea, sem, seo</i>
			1	t1848	15-12-17-16-02-16-02-25-17	30 (1)	—	—	—	—	<i>sem, seo</i>
435 (5)	4	ND	1	t270	14-44-13-12-17-17-17-17-23-18-17	121 (1)	—	—	—	—	<i>seb, sem, seo</i>
			3 (1)	t435	14-44-13-17-17-17-23-18-17	121 (1)	—	—	—	—	<i>sem, seo</i>
			1	t645	14-44-13-12-17-23-18-17	121 (1)	—	—	—	—	<i>seb, sem, seo</i>
Singletons	3	ND	1 (1)	t044	07-23-12-34-34-33-34	80 (1)	+	IVc	—	—	<i>etd, edinABC^d</i>
	3		2 (1)	t127	07-23-21-16-34-33-13	1 (1)	—	—	—	—	<i>sea, seb, seh, selk, selq</i>
	1		1	t4690	07-56-12-22-31-57-12	152 (1)	—	—	—	—	<i>edinABC</i>
	2		1 (1)	t4883	26-23-20-13-21-17-34-34-33-34	573 (1)	—	—	—	—	<i>sea, sec, sel, sem, seo</i>

^a PFGE was performed only for *agr-I* mutant isolates. The two reference strains corresponding to USA300-0114 isolates had PFGE pattern A. Isolates with PFGE patterns B and C showed 75.6% homology with those with PFGE pattern A and 78.1% homology with each other.

^b The number of foreign patients is in parentheses.

^c ND, not determined.

^d *edinABC*, epidermal cell differentiation inhibitor.

all of the isolates were susceptible to fluoroquinolones and kanamycin. The MRSA isolates with this pattern revealed the SCC*mec* IVc element. These characteristics matched those of a variant CA-MRSA USA300 clone described in Latin Amer-

ican hospitals (29) that has become established in South America and is endemic in hospital settings in some countries. Such a variant has also been described in Italy (23). We noticed that the tetracycline resistance rate was significantly lower in our

TABLE 2. Clinical features and susceptibility profile of PVL⁺ isolates^a

Parameter	<i>spa</i> -CC008		<i>spa</i> -CC433 ST30 (<i>n</i> = 6)	<i>spa</i> -CC435 ST121 (<i>n</i> = 5)	Singleton ST1 (<i>n</i> = 2), ST80, ST573, or ST152
	USA300-0114 (<i>n</i> = 7)	USA300 related (<i>n</i> = 17)			
Patient demographic data					
Mean age in yr (range)	50.7 (20–78)	23.7 (149 days-89)	24.6 (6–42)	21.3 (11–51)	26.4 (18–46)
No. (%) who required hospital admission	2 (28.6)	8 (47)	3 (50)	4 (80)	2 (40)
No. of days hospitalized (range)	11 (11 in both)	9.2 (1–24)	3.3 (2–5)	15.2 (1–41) ^b	6 (5–7)
No. (%) treated as outpatients	4 (57.1)	7 (41)	2 (33.3)		1 (20)
No. (%) treated in emergency room	1 (14.3)	2 (11.8)	1 (16.7)	1 (20)	2 (40)
No. (%) from abroad	2 (28.6)	4 (23.5)		1 (20)	3 (60)
No. (%) with SSTIs	7 (100)	15 (88.2)	6 (100)	2 (40)	5 (100)
Susceptibility profile ^a					
No. (%) <i>mecA</i> positive	7 (100)	13 (76)	2 (33)	0 (0)	1 (20)
Total resistance	Pen, Met, Ery, Kan, Flq	Pen		Pen	Pen
Variable resistance	Lin, MLS, Tob, Mup	Met, Ery, Lin, MLS, Gen, Tet	Pen, Met	Ery, Lin, MLS, Tet	Met, Kan, Tet, Fus, Rif, SXT

^a Antibiotics tested: Pen, penicillin; Met, methicillin; Ery, erythromycin; Lin, lincomycin; MLS, macrolides-lincosamides-streptogramin B; Kan, kanamycin; Tob, tobramycin; Tet, tetracycline; Mup, mupirocin; Flq, fluoroquinolone; Fus, fusidic acid; Rif, rifampin; Gen, gentamicin; SXT, cotrimoxazole.

^b One patient died within 24 h of admission.

series. PFGE pattern C was found in a single MRSA isolate that harbored the SCCmec IVa element and the AT repeat signature, lacking the *arcA* gene. The Spanish autochthonous population represents 75% (16/24) of the patients infected with these USA300-related isolates. One patient was from Ecuador, and the rest of the patients had no history of traveling to South America or other countries where the USA300 clone is prevalent.

Three other CA-MRSA isolates detected in Spanish patients with no report of any travel abroad were collected, a European ST80 clone ($n = 1$) and a Southwest Pacific ST30 clone ($n = 2$). Finally, the 13 remaining PVL-positive strains were MSSA harboring all of the *agr* alleles and so reflect a large diversity of genetic backgrounds. Most of them belonged to the ST121 ($n = 5$) and ST30 ($n = 3$) clones that have been widely detected across the world. We actually noticed that necrotizing pneumonia cases ($n = 3$) in our study were all associated with ST121 MSSA, which might indicate a higher virulence of this clone within this pathology.

As previously described in Europe, PVL genes were detected in strains with different genetic backgrounds (9, 28, 30, 35, 36). However, the epidemiology in Bilbao appeared unusual. First, contrary to all previously published European data, the most common PVL-positive CA-MRSA clone is not the European ST80-MRSA clone (8, 17, 20, 35, 36) but the USA300 clone and its Latin American variants. This is likely related to the specific and intensive population exchange between Spain and this continent. This specific epidemiology is worrying because of the enhanced virulence and transmissibility of this clone and the rapid spread that has recently occurred in the United States (24, 27, 31). Second, transatlantic import of USA300 strains has been previously described (21, 33) and seems to be the way they were acquired in the case series previously published in Spain (4, 5, 22). Our results demonstrated that domestic spread now seems to be the most prominent dissemination route in the Bilbao area. This is consistent with recent data from Denmark (21) or the United Kingdom (12), where only 26% and 13% of the USA300 cases were imported, respectively.

The reasons why the USA300 genetic background is dominant in the United States remain unclear. The presence of ACME in its genome has been proposed to be involved in this dominance (10, 11). In our series, we noticed that ACME-negative USA300 isolates were more prevalent than ACME-positive USA300 isolates and showed greater virulence with higher rates of hospitalization. These data argue that ACME alone is probably not sufficient to explain the properties of this clone.

Based on the extensive molecular data provided in this study, using one of the largest PVL-positive Spanish collections available, our results demonstrate that the USA300 clone was able to colonize and spread to become the most predominant PVL-positive *S. aureus* clone in a European autochthonous population. In Spain, it is likely that the USA300 strains are now resident in the community, but the reason for this specific epidemiology remains unclear, and it might be due to differences in predisposing conditions in the community and the high level of exchanges and migrants between Spain and Latin America. Infection control procedures must be employed to avoid spread in hospitals, not only in patients but also in

carriers. The emergence of these virulent strains strengthens the importance of rapid identification tools to detect and/or identify them. The different markers previously proposed (e.g., AT repeat, *arcA*, etc.) are not robust enough, as confirmed by our results. Moreover, combined with epidemiological data mentioned before, our results suggest that the ST80 clone might not be the European counterpart of USA300 in Europe (13, 21); rather, it might be rapidly outcompeted by USA300. This suggests that active and rapid monitoring of the temporal and geographical incidence of, as well as the epidemiology and disease burden associated with, these strains is required in Spain and throughout Europe.

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