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Rapid Detection of *Staphylococcus aureus* Panton-Valentine Leukocidin in Clinical Specimens by Enzyme-Linked Immunosorbent Assay and Immunochromatographic Tests[▽]

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Staphylococcus aureus **strains producing Panton-Valentine leukocidin (PVL) have been epidemiologically linked to specific human infections. To evaluate immunological tests that may be used to diagnose infections with PVL-producing strains, we prospectively collected pus, respiratory tract specimens, and joint fluid specimens from which *S. aureus* had been isolated in clinical laboratories in six countries. An enzyme-linked immunosorbent assay (ELISA) and an immunochromatographic test (ICT) targeting LukS-PV were performed directly with clinical samples for the detection of PVL. The same tests were applied to *S. aureus* culture supernatants. The corresponding *S. aureus* isolates were characterized by PCR for the presence of the PVL locus (*lukS-PV* and *lukF-PV*) and the *mecA* gene. A total of 185 samples from 144 skin infections, 23 bone and joint infections, and 18 lower respiratory tract infections were analyzed. By PCR, 72/185 *S. aureus* isolates were PVL locus positive (PVL$^+$); 28 of these were also *mecA* positive. PVL was detected in the supernatants of all PVL$^+$ strains by both ELISA and an ICT, while no signal was observed with PVL-negative strains. The PVL concentrations in human clinical samples that grew PVL$^+$ strains ranged from 0 to 399 μg/ml by ELISA. By the use of 0.015 μg/ml of PVL as a cutoff value, PVL was detected in 65/72 (90%) of the clinical samples by ELISA. The sensitivity and specificity of the ELISA test were 90% and 100%, respectively. By the ICT, PVL was detected in 57/72 (79%) of the samples, and the sensitivity and specificity of ICT were 79% and 100%, respectively. PVL is expressed by *S. aureus* during human infection, and a PVL-specific ELISA and ICT could be reliable tests for the diagnosis of infections caused by PVL-producing strains.**

Staphylococcus aureus is an important human pathogen whose pathogenicity largely depends on extracellular virulence factors. One of these exoproteins, Panton-Valentine leukocidin (PVL), is produced by several community-acquired methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus* (CA-MRSA and CA-MSSA, respectively) clones currently spreading throughout the world (27).

Historical publications show that PVL targets cells of the human immune system, such as polymorphonuclear neutrophils (PMNs), monocytes, and macrophages (26). *In vitro*, PVL forms pores on human and rabbit PMNs and monocytes that cause cytokine release and cell death by apoptosis or necrosis (13, 18). In rabbit models, PVL provokes a dose-dependent skin necrosis (7, 30); bacterial persistence in bone; and a rapid local extension of osteomyelitis (6), severe lung necrosis, pulmonary edema, alveolar hemorrhage, and death (10).

Isolates of *S. aureus* harboring PVL genes have been epidemiologically linked to specific human *S. aureus* manifestations: not only to primary skin and soft tissue disease but also to severe necrotizing pneumonia and severe bone and joint infections (4, 11, 14, 19, 21, 24). In certain countries, such as the United Kingdom and France, PVL is now detected in clinical practice, and treatment regimens may be adjusted on the basis of the presence or the absence of PVL (12, 15, 17). The adjunctive use of antibiotics that suppress toxin production, such as clindamycin, linezolid, and rifampin, and intravenous immunoglobulin is advocated for the treatment of severe and invasive infections caused by PVL-producing strains.

To date, the diagnosis of infection due to PVL-producing strains is mainly performed by PCR of colonies for the detection of PVL genes, while the latex agglutination assay and matrix-assisted laser desorption ionization–time of flight mass

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spectrometry method should be performed with colonies (3, 19, 23). However, the results of these methods of PVL detection may not correlate with *in vivo* production.

In a previous study, we have shown that PVL can be detected in pus from a skin abscess caused mainly by PVL-producing methicillin-susceptible *S. aureus* (1). The objective of this study was to determine whether the PVL produced in clinical specimens from PVL gene-positive *S. aureus* infections could be detected by a specific enzyme-linked immunosorbent assay (ELISA) or immunochromatographic test (ICT), regardless of the genetic background and methicillin susceptibility of the PVL-producing strains.

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MATERIALS AND METHODS

Sample inclusion. The study was conducted in accordance with the guidelines of the ethical committees of the six participating hospitals. In each hospital, specimens from human infections that were collected as part of the routine management of patients with *S. aureus* infections were prospectively stored at −20°C in Eppendorf tubes over a period of 6 months. Only clinical specimens with a volume of 300 μl or greater were retained. Samples were shipped to Lyon, France, on dry ice for PVL detection.

In total, 144 pus samples from *S. aureus* skin infections were obtained and analyzed: 88 from France, 16 from Australia, 13 from Algeria, 10 from Singapore, 10 from the United States, and 7 from Greece. In Lyon, France, we collected an additional 18 clinical specimens from patients with pneumonia (4 pleural fluid, 3 bronchoalveolar lavage [BAL], 8 tracheal aspirate, and 3 sputum specimens) and 23 specimens from patients with bone and joint infections (14 pus specimens from patients with osteitis and 9 joint fluid specimens).

Isolate analysis. *S. aureus* was identified on the basis of the colony and cell morphologies, the results of coagulase testing with rabbit plasma (bioMérieux, Marcy l'Etoile, France), and the results of tests with the Slidex Staph Plus kit (bioMérieux). Genomic DNA was extracted from the staphylococcal cultures by a standard procedure (20) and was used as the template for amplification by using primers previously shown to be specific for the PVL genes (*lukS-PV* and *lukF-PV*, which form the operon for the PVL locus) and the *mecA* gene (which codes for methicillin resistance) (19, 22). Multilocus sequence types (STs) and *spa* types were determined for PVL locus-positive (PVL^+) isolates, as described previously (27).

Since the optimal yield of PVL is obtained by cultivation of strains in casein-casein-yeast extract (CCY) broth, (3% [wt/vol] yeast extract, 2% Bacto Casamino Acids, 2% sodium pyruvate, 0.25% $NaHPO_4$, and 0.042% KH_2PO_4 [pH 7.0]), 5 ml of CCY medium was inoculated with each isolate (five colonies from an overnight culture on blood agar) and incubated at 37°C with vigorous shaking for 18 h (31). After centrifugation, the culture supernatants were sterilized by 1 h of heating at 94°C, followed by cooling at +4°C on ice for 5 min and storage at +4°C until PVL detection was performed.

Analysis of clinical specimens. All clinical specimens culture positive for *S. aureus* (regardless of the *luk-PV* or *mecA* status of the corresponding isolates) were thawed to +4°C, vortexed for 1 min, sterilized by 1 h of heating at 94°C and then cooling at +4°C on ice for 5 min, and centrifuged at 10,000 × *g* for 5 min. The supernatants were used for PVL detection and quantification.

PVL ELISA. PVL detection and quantification were performed by an antibody-sandwich ELISA targeting LukS-PV with solid-phase mouse anti-LukS-PV monoclonal antibody and revelation by horseradish peroxidase-conjugated rabbit polyclonal $F(ab')_2$ anti-LukS-PV from the R&D Immunoassays department of bioMérieux, as described by Badiou et al. with few modifications (1). Briefly, the wells of microtiter plates (Sigma, Saint Quentin Fallavier, France) were coated with anti-LukS-PV monoclonal antibody in phosphate-buffered saline (PBS) overnight at room temperature. Unbound monoclonal antibody was washed out twice with a solution of PBS-Tween (0.05%) (PBST), and nonspecific binding sites were blocked with a PBST-milk (5%) solution for 30 min at 37°C. The plates were further washed another tree times with PBST before standard recombinant LukS-PV dilutions (from 0.005 to 0.8 μg/ml in PBST-milk [1%]) or samples (undiluted or diluted in PBST-milk [1%]) were added to duplicate wells for 1 h 30 min at 37°C (2). After three washes with PBST, horseradish peroxidase-conjugated rabbit polyclonal $F(ab')_2$ anti-LukS-PV was added. Subsequently, the microplates were incubated for 1 h 30 min at 37°C and washed tree times with PBST, before revelation by adding the enzyme substrate (tetramethylbenzidine). After a 30-min incubation in the dark at room temperature, the reaction was stopped by adding H_2SO_4 solution. The plates were read at 450 nm in a model 680 microplate reader (Bio-Rad). No signal was detected with recombinant LukF-PV, HlgA, HlgB, HlgC, LukE, or LukD.

ICT. The ICT device was developed by the R&D Immunoassays department of bioMérieux. It consisted of a chromatography strip, a separator, and an absorbent pad, all housed in a cassette. The cassette contains a nitrocellulose membrane on which monoclonal mouse anti-LukS-PV antibody is adsorbed. A second mouse anti-LukS-PV antibody is coupled to red-dyed polystyrene microspheres, which are dried onto an inert fibrous support. The test was performed according to the instructions of the R&D Immunoassays department of bioMérieux. Briefly, 70 μl of sample was applied to the sample well of the strip, and the sample migrated by capillary action along the nitrocellulose membrane. If the sample contains LukS-PV, the LukS-PV present in the sample binds to the anti-LukS-PV-conjugated antibodies and the resulting antigen-antibody complexes are captured by the immobilized monoclonal mouse anti-LukS-PV antibody, forming the test line. The test was read at 30 min, and the result was interpreted by noting the presence or the absence of a visually detectable red line. A positive test result was indicated by the detection of a red line in the test line region. A negative test result was indicated by the absence of the red line.

Statistical analysis. The Mann-Whitney U test (because of the rejection of normality) and receiver-operator-characteristic (ROC) curve analyses were performed with SPSS software, version 16.0 (SPSS Inc., Chicago, IL), and were used to identify differences in PVL titers between groups of strains or samples that were PVL^+ and PVL negative (PVL^-). The level of statistical significance was set at a P value of <0.05.

RESULTS

Isolates analysis. One hundred eighty-five *S. aureus* isolates recovered from patients with infections were analyzed for the presence of the PVL locus and *mecA* genes by PCR (Table 1). Seventy-two (39%) were positive for the PVL locus. *mecA* was detected in 40 (22%) of the isolates; 12/40 (30%) isolates were PVL^- and 28/40 (70%) were PVL^+. All PVL^+ *S. aureus* isolates were analyzed by multilocus sequence typing (MLST), and their *spa* types were determined. The 72 PVL^+ isolates belonged to 20 different STs, corresponding to 36 different *spa* types (Table 1).

Extracellular PVL production was evaluated by analyzing the supernatants for all 185 isolates. By ICT, PVL was detected in all 72 supernatants derived from the PVL^+ isolates, while no signal was observed with the 113 supernatants from the PVL^- strains.

The quantity of PVL produced *in vitro* was determined by ELISA. The level of PVL in the supernatants varied among isolates, ranging from 0.09 to 14.57 μg/ml (median, 1.28 μg/ml). There was no difference in the levels of PVL production *in vitro* between *mecA*-positive ($mecA^+$) and *mecA*-negative ($mecA^-$) isolates (for the $mecA^+$ isolates the median was 1.17 μg/ml and the range was 0.24 to 14.57 μg/ml, and for the $mecA^-$ the median was 1.69 μg/ml and the range was 0.09 to 14.40 μg/ml; $P = 0.33$). In order to examine whether isolates belonging to specific STs were higher-level PVL producers, the STs with nine and more isolates, i.e., strains belonging to ST8, ST30, ST80, or ST121, were compared (Fig. 1). The PVL levels in the supernatants of ST8 isolates (median, 5.05 μg/ml; range, 3.56 to 14.57 μg/ml) were similar to the PVL levels in the supernatants of ST121 isolates (median, 7.98 μg/ml; range, 0.26 to 14.30 μg/ml) ($P = 0.81$), but the PVL levels in the supernatants of both ST8 and ST121 isolates were higher than those in the supernatants of ST80 isolates (median, 0.75 μg/ml; range, 0.09 to 3.55 μg/ml; $P = 0.007$ and 0.003, respectively)

TABLE 1. Clinical samples and isolates used in this study and PVL detection by specific ICT and ELISA[a]

Infection type (no. of isolates)	No. of isolates	ST	*spa* type	PVL	*mecA*	PVL production (no. positive/total no.)			
						Isolates		Clinical specimens	
						ICT	ELISA	ICT	ELISA
Skin infections (144)	1	1	t3688	+	−	1/1	1/1	1/1	1/1
	1	1	t127	+	−	1/1	1/1	1/1	1/1
	1	5	t311	+	−	1/1	1/1	1/1	1/1
	2	8	t008	+	−	2/2	2/2	2/2	2/2
	8	8	t008	+	+	8/8	8/8	5/8	5/8
	1	15	t084	+	−	1/1	1/1	1/1	1/1
	1	22	t005	+	−	1/1	1/1	0/1	1/1
	1	25	t4844	+	−	1/1	1/1	1/1	1/1
	2	26	t078	+	−	2/2	2/2	2/2	2/2
	1	30	t019	+	−	1/1	1/1	1/1	1/1
	2	30	t021	+	−	2/2	2/2	2/2	2/2
	1	30	t090	+	−	1/1	1/1	1/1	1/1
	1	30	t242	+	−	1/1	1/1	1/1	1/1
	1	30	t318	+	−	1/1	1/1	0/1	1/1
	1	30	t363	+	−	1/1	1/1	1/1	1/1
	1	30	t605	+	−	1/1	1/1	1/1	1/1
	1	80	t042	+	+	1/1	1/1	1/1	1/1
	11	80	t044	+	+	11/11	11/11	8/11	10/11
	3	80	t044	+	−	3/3	3/3	3/3	3/3
	1	88	t729	+	−	1/1	1/1	1/1	1/1
	1	88	t1598	+	−	1/1	1/1	1/1	1/1
	1	88	t3155	+	−	1/1	1/1	1/1	1/1
	1	88	t4333	+	−	1/1	1/1	1/1	1/1
	2	93	t202	+	+	2/2	2/2	2/2	2/2
	1	120	t3616	+	−	1/1	1/1	1/1	1/1
	2	121	t314	+	−	2/2	2/2	2/2	2/2
	1	121	t582	+	−	1/1	1/1	1/1	1/1
	1	121	t645	+	−	1/1	1/1	1/1	1/1
	3	121	t3616	+	−	3/3	3/3	2/3	2/3
	1	121	t4333	+	−	1/1	1/1	1/1	1/1
	1	152	t355	+	−	1/1	1/1	1/1	1/1
	1	152	t5227	+	−	1/1	1/1	1/1	1/1
	1	217	t4466	+	−	1/1	1/1	0/1	1/1
	1	291	t1614	+	−	1/1	1/1	0/1	1/1
	1	378	t3651	+	−	1/1	1/1	1/1	1/1
	1	728	t160	+	+	1/1	1/1	1/1	1/1
	1	772	t657	+	+	1/1	1/1	1/1	1/1
	1	1301	t3666	+	−	1/1	1/1	1/1	1/1
	5	ND	ND	−	+	0/5	0/5	0/5	0/5
	75	ND	ND	−	−	0/75	0/75	0/75	0/75
Pneumonia (18)	1	30	t021	+	−	1/1	1/1	0/1	0/1
	4	80	t044	+	+	4/4	4/4	2/4	3/4
	1	121	t5228	+	−	1/1	1/1	0/1	1/1
	4	ND	ND	−	+	0/4	0/4	0/4	0/4
	8	ND	ND	−	−	0/8	0/8	0/8	0/8
Bone/joint infection (23)	1	30	t302	+	−	1/1	1/1	1/1	1/1
	1	152	t355	+	−	1/1	1/1	1/1	1/1
	3	ND	ND	−	+	0/3	0/3	0/3	0/3
	18	ND	ND	−	−	0/18	0/18	0/18	0/18

[a] STs were determined by multilocus sequence typing. PVL locus genes, which encode the Panton-Valentine leukocidin, and the *mecA* gene, which codes for methicillin resistance, were detected by PCR of the corresponding genes. The cutoff value for the PVL ELISA was 0.015 μg/ml; −, negative; +, positive; ND, not determined.

and ST30 isolates (median, 0.44 μg/ml; range, 0.10 to 14.40 μg/ml; $P = 0.005$ and $P = 0.003$, respectively). The PVL levels in the supernatants of ST80 isolates and ST30 isolates were similar ($P = 0.054$).

Clinical specimen analysis. The clinical specimens prospectively collected from patients with *S. aureus* infections were blindly analyzed in the Lyon laboratory for the presence of PVL.

By ICT, PVL was detected in 57/72 *S. aureus* PVL$^+$ specimens (79%) (Tables 1 and 2). These samples were derived from 53/64 (83%) pus samples from skin infections, 2/2 bone abscess samples, and 2/6 pulmonary samples (Table 1). The two positive pulmonary specimens corresponded to pleural fluids, while the negative specimens comprised one pleural fluid specimen, one BAL specimen, one sputum specimen, and one tracheal aspirate specimen. All 113 *S. aureus* PVL$^-$ spec-

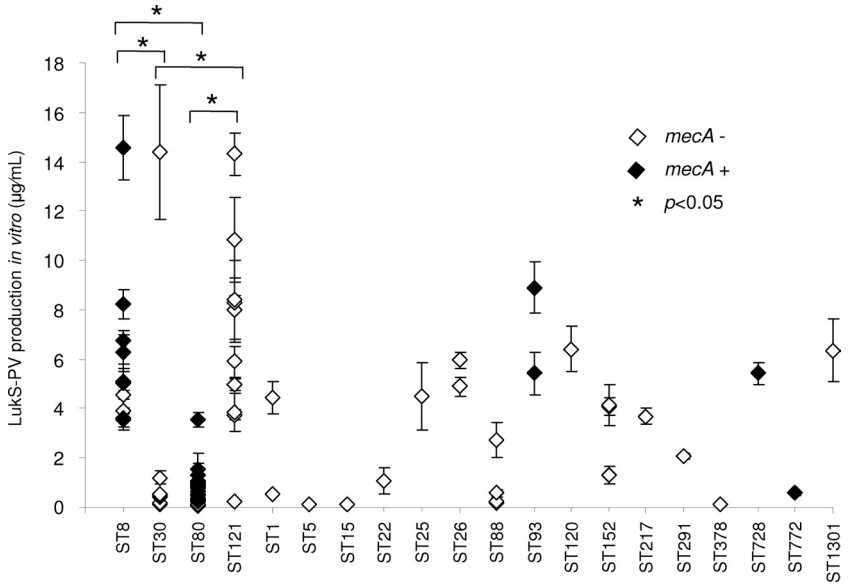

FIG. 1. Variability of PVL production among clinical isolates of *S. aureus* with a given genetic background. Isolates were grouped according to their multilocus sequence type. ST8 MRSA strains correspond to the USA300 CA-MRSA clone, ST80 strains correspond to the European CA-MRSA clone, and ST93 strains correspond to the Queensland ST93 CA-MRSA clone, while ST121 strains correspond to a predominant methicillin-sensitive *S. aureus* PVL$^+$ clone spreading in Europe (20, 25, 29). The level of PVL production *in vitro* was measured by ELISA. The results are expressed as the means of three experiments ± standard errors of the means. *, $P < 0.05$, Mann-Whitney U test.

imens were negative by ITC. Thus, for clinical specimens, the sensitivity and the specificity of ICT were 79% and 100%, respectively (Table 2).

By ELISA, the PVL concentrations in the 72 *S. aureus* PVL$^+$ samples had a median of 0.42 μg/ml (range, 0 to 399 μg/ml), as shown in Fig. 2. The PVL concentrations had medians of 0.54 μg/ml (range, 0 to 399 μg/ml) in pus from skin infections, 0.11 μg/ml (range, 0 to 0.33 μg/ml) in pulmonary samples, and 128.4 μg/ml (range, 0.10 to 256 μg/ml) in bone abscess samples.

PVL was not detected by ELISA in seven human samples harboring PVL$^+$ strains. Those samples were also negative for PVL by ICT. Eight *S. aureus* PVL$^+$ samples negative for PVL by ITC had detectable values by ELISA: median, 0.29 μg/ml (range, 0.021 to 1.049 μg/ml). There was no difference in the PVL levels in pus from skin infections related to whether the corresponding PVL$^+$ *S. aureus* infecting isolates were *mecA*$^+$ or *mecA*$^-$ ($P = 0.40$).

Likewise, the PVL levels in the skin abscess specimens were not related to a certain genetic background (ST; i.e., ST8, ST80, ST30, and ST121) (*P* value range, 0.07 to 0.68) (Fig. 2). PVL was detected in only two samples that grew *S. aureus* PVL$^-$ strains; these were two pus samples from skin infections, in which we detected 0.005 μg/ml of PVL, which corresponds to the detection limit of our ELISA. The area under the ROC curves was 0.97 (95% confidence interval, 0.93 to 0.99; $P < 0.001$), and 0.015 μg/ml was determined to be the cutoff value for the ELISA. By use of this cutoff value, PVL was detected in 65/72 (90%) of the *S. aureus* PVL$^+$ clinical specimens. They corresponded to 59/64 (92%) of the samples of pus from *S. aureus* PVL$^+$ skin infections (39/40 [98%] from France and 20/24 [83%] from other countries; $P = 0.038$), 2/2 *S. aureus*

TABLE 2. Results obtained by ICT and ELISA with the clinical specimens

Test and isolate source	No. of isolates with the following result/total no. tested:				Sensitivity (%)	Specificity (%)
	True positive	False positive	True negative	False negative		
ITC						
Total	57/72	0/113	113/113	15/72	79	100
Skin infection	53/64	0/80	80/80	11/64	83	100
Pneumonia	2/6	0/12	12/12	4/6	NA[a]	NA
Bone and joint infection	2/2	0/21	21/21	0/2	NA	NA
ELISA						
Total	65/72	0/113	113/113	7/72	90	100
Skin infection	59/64	0/80	80/80	5/64	92	100
Pneumonia	4/6	0/12	12/12	2/6	NA	NA
Bone and joint infection	2/2	0/21	21/21	0/2	NA	NA

[a] NA, not applicable due to the small number of corresponding samples.

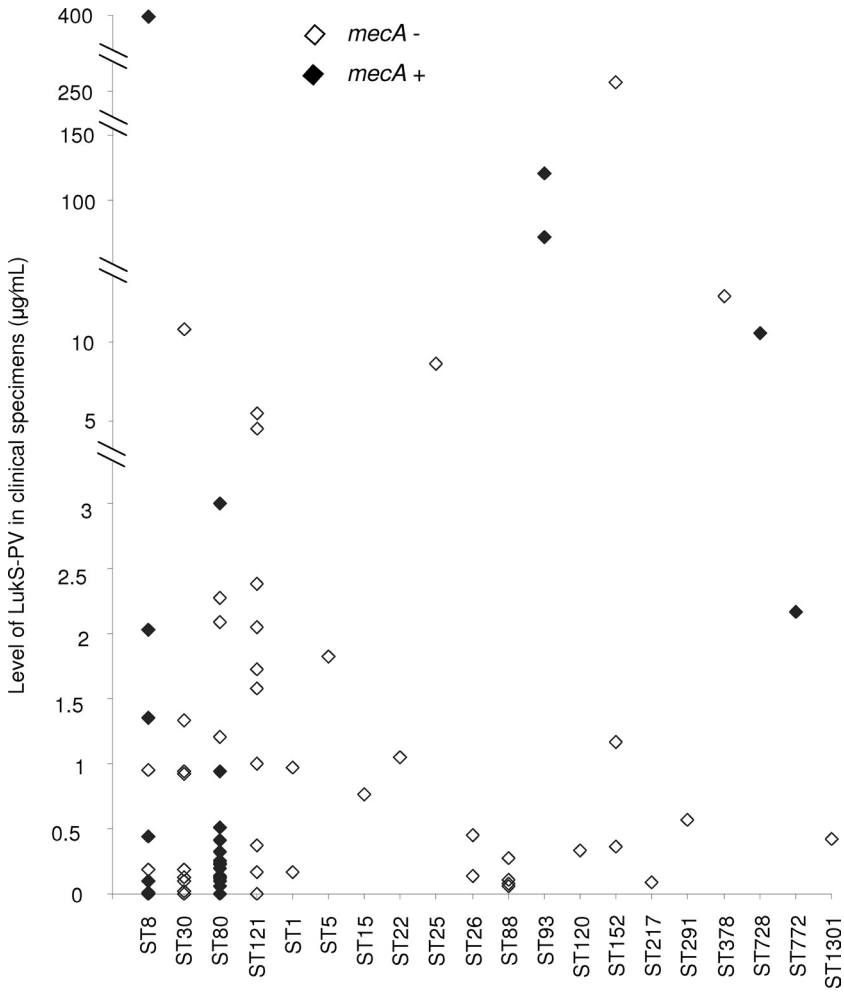

FIG. 2. Variability of PVL concentration among clinical specimens containing *S. aureus* isolates with a given genetic background. Isolates were grouped according to their multilocus sequence types, while the PVL concentrations in samples from which the corresponding strains were isolated were measured by ELISA.

PVL^+ bone abscess samples, and 4/6 (67%) of the pulmonary samples from patients with *S. aureus* PVL^+ pneumonia. The sensitivity and the specificity of the ELISA were 90% and 100%, respectively (Table 2). The results observed by ELISA and ICT were strongly correlated ($P < 0.001$). When we compared the PVL concentrations in clinical specimens to those produced by the corresponding isolates *in vitro*, we did not find a correlation (Pearson correlation test, $P = 0.20$). However, there were very good correlations between the presence of the genes by PCR, *in vivo* production (of any concentration), and *in vitro* production ($P < 0.001$).

DISCUSSION

The aim of the study described here was to determine whether the PVL produced in clinical specimens obtained from patients with PVL gene-positive *S. aureus* infections could be detected by a specific ELISA or ICT.

For this purpose, we prospectively collected and analyzed clinical specimens and the corresponding *S. aureus* strains isolated from patients with infections in which PVL was suspected to play a role in the pathophysiology, i.e., skin infections, pneumonia, and bone and joint infections (4, 11, 14, 19, 24). Samples were collected from six different countries to obtain strains with more diverse genetic backgrounds (27).

We collected 185 pairs of *S. aureus* strains and clinical specimens. Thirty-nine percent of the strains carried the genes for the PVL locus. All these strains produced PVL *in vitro*, while no PVL was detected in the culture supernatants of PVL^- strains. On the basis of these results, we concluded that the sensitivities and the specificities of both the ICT and the ELISA methods for the detection of PVL-producing strains were equivalent to those of the PCR method. MLST and *spa* typing analyses showed that the PVL^+ strains were distributed among 36 different clones. Some of the PVL^+ strains belonged to predominant CA-MRSA clones ST8 (the USA300 clone) (25), ST80 (the European ST80 CA-MRSA clone), and ST93 (the Queensland ST93 CA-MRSA clone) (29). The levels of PVL production *in vitro* varied between strains, even within specific genetic backgrounds. This interstrain variability has previously been reported for ST8 strains (16), and we also report the same observation for other STs, such as ST80 and

ST93. We noted that certain STs were stronger PVL producers *in vitro*: ST8 PVL$^+$ strains corresponding to CA-MRSA clone USA300 and ST121 PVL$^+$ strains corresponding to a predominant MSSA clone spreading in Europe (20) were the two strongest PVL producers, while ST80 PVL$^+$ strains (the European CA-MRSA clone) produced 7-fold less PVL. The origin of the variation in PVL production is not known. Since CA-MRSA frequently carries the PVL genes (29), we examined whether *mecA*$^+$ strains produced more PVL than *mecA*$^-$ strains. However, no differences in the PVL concentrations were observed between *mecA*$^+$ and *mecA*$^-$ strains either in the global analysis or in the analysis restricted to STs. Therefore, it seems unlikely that *mecA* has an impact on PVL production or influences the fitness of strains by modulation of PVL expression. The association between the *mecA* and the PVL locus genes in CA-MRSA remains to be elucidated.

Since all of the PVL$^+$ strains produced PVL *in vitro*, we expected that we would detect PVL in the corresponding specimens. We detected PVL in the great majority of the clinical samples using the two immunological methods (ICT and ELISA). For ICT and ELISA, the sensitivities were 79% and 92%, respectively, while the specificities were 100% for both methods. We did not detect PVL in clinical specimens that grew PVL$^+$ strains by ICT when the strains were PVL negative by ELISA. ICT appeared to be slightly less sensitive than ELISA. The difference in sensitivity between these two methods may be related to the use of different antibodies: two monoclonal antibodies for ICT versus one monoclonal antibody and polyclonal antibodies for ELISA. When we examined the results for the skin infection specimens, we noted that isolates in which no PVL production was detected were statistically more commonly from outside of Lyon. This suggests that storage and transportation may have caused PVL deterioration. Moreover, clinical specimens contain proteases produced by *S. aureus* and released by host cell alteration that may damage the PVL if the sample is not rapidly examined. All pulmonary specimens were collected in France. Only two respiratory samples were PVL negative by ELISA. One of these patients had necrotizing pneumonia and the blood culture grew PVL$^+$ *S. aureus*, while the pleural effusion, from which our specimen was obtained, was culture negative. In the second patient, the BAL fluid specimen tested grew PVL$^+$ *S. aureus* at a colony count of less than 10^4/ml. These two cases suggest that PVL detection in clinical specimens by ELISA and ICT is dependent on the presence of a sufficient density of PVL-producing bacterial cells.

Using ELISA, we were able to quantify the level of PVL in human samples with PVL$^+$ isolates. The PVL concentrations varied from 0 to 399 μg/ml. Previous studies have reported that a toxin concentration of 0.050 μg/ml induces human polymorphonuclear leukocyte activation (18), one of 0.33 μg/ml causes human polymorphonuclear leukocyte apoptosis (13), and an intradermal injection of 0.3 μg causes local inflammation and necrosis in a rabbit experimental model (7, 30). Ninety percent of the PVL$^+$ specimens contained PVL at concentrations toxic for human leukocytes. Our results confirm the previous observation that PVL$^+$ *S. aureus* strains produce PVL at concentrations that will likely induce a host inflammatory response in the infected tissue during human infection (1).

Are some genetic backgrounds of PVL$^+$ strains higher-level producers of PVL *in vivo*, as observed *in vitro* (16)? Focusing on skin infections, we did not find an association between specific genetic backgrounds and PVL concentrations. Furthermore, PVL concentrations were not dependent on the presence or the absence of *mecA* within the genetic backgrounds. In addition, the PVL concentrations from specimen-isolate pairs did not correlate; one could produce high levels of PVL, while the other produced only small amounts. It is probable that factors such as the type and extent of the infection, the body site, and patient immunity factors influence the level of PVL produced during the course of infection (8). Specimen deterioration due to transportation may also have confounded our results. A reliable comparison of the *in vitro* and *in vivo* findings would entail quantitative cultures of specimens and correlation to the number of bacteria present. That was not attempted in this study. The difficulty in correlating the two entities was previously discussed by Hamilton et al. (16).

Epidemiological data from different groups suggest that PVL$^+$ *S. aureus* strains are associated with some of the severe forms of skin infection (9, 19, 24, 32), bone and joint infections (4, 11), and pneumonia (14, 15). For these infections, experimental data support the role of PVL in the severity of disease (5, 6, 10, 28). The early diagnosis of PVL-positive *S. aureus* infections by these immunological methods will allow physicians to rapidly identify PVL-associated diseases. This might become important if subsequent studies demonstrate that the inhibition of PVL production and action results in improved clinical outcomes (12, 15, 17).

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