

## Frequency of Pantone-Valentine Leukocidin-Producing Methicillin-Sensitive *Staphylococcus* Strains in Patients with Complicated Skin and Soft Tissue Infection in Bronx, New York<sup>∇</sup>

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***lukF-PV* was present in 36% of skin and soft tissue infection (SSTI)-derived methicillin-susceptible *Staphylococcus aureus* (MSSA) strains and comprised six distinct clones, which contained fewer enterotoxin genes than strains without *lukF-PV*. Clinical presentations and outcomes of *lukF-PV*<sup>+</sup> methicillin-resistant *S. aureus* (MRSA) and MSSA SSTIs were comparable. In multivariable analysis, the presence of *lukF-PV* remained a significant predictor for incision and drainage among MSSA strains.**

In the United States, Pantone-Valentine leukocidin (PVL), a pore-forming cytotoxin, is investigated mostly in the emerging community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) clone USA300 (10). PVL contribution to virulence is disputed, as summarized by Schlievert (6). PVL is also produced by strains other than USA300, and levels vary considerably among individual strains (2, 8). Some methicillin-susceptible *S. aureus* (MSSA) strains produce large amounts of PVL, which has been associated with more-severe skin lesions in a murine model (8). In this study, we compared the prevalences of PVL in MSSA and MRSA strains from patients with skin and soft tissue infections (SSTIs) and compared the clinical presentations. *S. aureus* isolates from wound ( $n = 108$ ) and blood ( $n = 99$ ) were collected by the microbiology laboratory of Montefiore Medical Center as described previously (9). Detection of enterotoxin genes (staphylococcal enterotoxin A [SEA], SEB, SEC, and toxic shock syndrome toxin [TSST] genes), multilocus sequence typing (MLST), and *spa* typing were done in the context of a previously published study (9). For this study, detection of PVL in MSSA isolates was performed by real-time PCR targeting the *lukF-PV* gene (7). Also, a retrospective chart review was performed on all 104 patients from whom wound isolates had been collected and 11 patients from whom PVL-producing *S. aureus* strains were cultured from blood. Information on demographic characteristics, clinical presentation, antibiotic therapy, complications, and outcomes was obtained. Fever was defined as greater than 38.0°C and leukocytosis as  $\geq 12.0$  K/ $\mu$ l. Health care-associated (HA) risk factors included end-stage renal disease (ESRD) on dialysis, hospitalization 6 months prior to admission, history of

*S. aureus* infection, and nursing home residence. The Wilcoxon rank-sum test was used to compare continuous variables, and the  $\chi^2$  or Fisher exact test was used to compare proportions. To assess the outcome variable of incision and drainage, selected predictor variables whose *P* values were less than 0.20 in univariate analysis were introduced into a multivariate logistic regression model for further analysis.

Chart review determined that 101 wound isolates were derived from patients with SSTIs (46 on extremities, 55 from other body parts). Clinical data from 4 patients were not retrievable, and 3 wound isolates were excluded as they were grown from infected bone biopsy specimens and an infected vascular graft. MSSA and MRSA strains were isolated in 57% and 43% of SSTI cases, respectively. A significant percentage of SSTI-associated MSSA strains (36%) and most MRSA strains (77%) carried the *lukF-PV* gene (Table 1). A significantly higher proportion of *S. aureus* strains (both MSSA and MRSA strains) that were derived from SSTIs than were derived from blood harbored the *lukF-PV* gene (53% versus 11%, respectively;  $P < 0.001$  by chi-square test). The majority (9 of 11) of *lukF-PV*<sup>+</sup> blood isolates were MRSA strains derived from patients with concomitant SSTIs. Only 2 of 45 MSSA strains isolated from blood contained the *lukF-PV* gene.

Patients infected with *lukF-PV*<sup>+</sup> *S. aureus* strains were significantly younger than those infected with *S. aureus* strains lacking *lukF-PV* (33.4 and 44.7 years, respectively;  $P = 0.02$ ) and had fewer hospitalizations within the prior 6 months (12 of 54 [22%] and 20 of 46 [43%], respectively;  $P = 0.02$ ).

A comparison of patients with SSTIs caused by MRSA *lukF-PV*<sup>+</sup> or MSSA *lukF-PV*<sup>+</sup> strains demonstrated similar lengths of hospital stay (median lengths of 6 and 4 days, respectively;  $P = 0.59$ ), proportions of complications by osteomyelitis (6% and 5%, respectively;  $P = 0.85$ ), and numbers of strains associated with bacteremia (3 MSSA strains and 2 MRSA strains). The percentage of patients that were treated by incision and drainage tended to be higher in those with SSTIs caused by

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TABLE 1. Baseline demographic and clinical characteristics<sup>a</sup>

Characteristic <sup>b</sup>	MSSA strains (n = 58)		MRSA strains (n = 43)		P value <sup>c</sup>
	PVL <sup>-</sup>	PVL <sup>+</sup>	PVL <sup>-</sup>	PVL <sup>+</sup>	
PVL status	37/58 (64)	21/58 (36)	10/43 (23)	33/43 (77)	0.001
Median age (yr)	39.5 (0–77)	34 (4–65)	68.5 (27–87)	29.5 (0.9–87)	0.303, 0.012*
Male sex	23/37 (62)	15/21 (71)	3/10 (30)	13/33 (39)	0.005
Race					
Hispanic	17/36 (47)	12/18 (67)	2/10 (20)	10/27 (37)	0.074
African-American	14/36 (39)	4/18 (22)	4/10 (40)	13/27 (48)	0.725
Caucasian	5/36 (14)	2/18 (11)	4/10 (40)	4/27 (15)	0.420
Patient risk factor					
NHR	0/37 (0)	0/21 (0)	3/10 (30)	2/33 (6)	0.012
Diabetes	11/37 (30)	3/21 (14)	3/10 (30)	6/33 (18)	0.704
ESRD/dialysis	3/37 (8)	0/21 (0)	4/10 (40)	1/33 (3)	0.280, 0.007*
HIV	3/37 (8)	3/21 (14)	0/10 (0)	2/33 (6)	0.461
Malignancy	4/37 (11)	0/21 (0)	2/10 (20)	2/33 (6)	0.720
Device-related infection	3/37 (8)	2/21 (9)	5/10 (50)	1/33 (3)	0.521, 0.001*
HA infection in past 6 mo	11/36 (31)	2/21 (10)	9/10 (90)	10/33 (30)	0.031
SA infection in past 6 mo	1/36 (3)	2/21 (9)	1/10 (10)	8/33 (24)	0.027
Antibiotics in last month	8/37 (22)	5/21 (24)	4/10 (40)	9/33 (27)	0.374
Presentation of SSTI					
Extremity and buttock	20/37 (54)	19/21 (43)	3/10 (30)	14/33 (42)	0.400
Fever of >100.4°F at Dx	9/36 (25)	2/21 (9)	3/10 (30)	8/33 (24)	0.453
Median WBC at Dx (range)	8.4 (3.2–48)	10.9 (6.3–21)	9.5 (1.3–24)	11.5 (3.7–30.1)	0.485
Treatment and outcome					
HA infection	23/37 (62)	7/21 (33)	9/10 (90)	18/33 (55)	0.267, 0.035*
Median LOS, days (range)	9 (0–134)	2 (0–33)	11.5 (1–32)	4 (0–68)	0.442
Incision and drainage	21/37 (57)	17/21 (81)	7/10 (70)	23/33 (70)	0.652
Osteomyelitis	4/34 (12)	1/21 (5)	4/10 (40)	2/32 (6)	0.526, 0.021*
Death	1/37 (3)	0/21 (0)	1/10 (10)	1/33 (3)	0.573
Appropriate Abx given	9/36 (25)	3/21 (14)	10/10 (100)	27/33 (81)	0.000
Median no. of SE (range)	6 (2–9)	4 (0–8)	5 (1–9)	3 (0–6)	0.002, 0.008*
SEA <sup>+</sup>	3/37 (8)	0/21 (0)	0/13 (0)	0/33 (0)	0.260
SEB <sup>+</sup>	5/37 (13)	0/21 (0)	0/10 (0)	1/33 (3)	0.233
TSST <sup>+</sup>	5/37 (13)	0/21 (0)	0/13 (0)	0/33 (0)	0.070
mecA IV	0/37 (0)	0/21 (0)	13/13 (100)	33/33 (100)	0.000

<sup>a</sup> Results are given as numbers (%) of strains with the characteristic, except where indicated.

<sup>b</sup> NHR, nursing home residence; SA, *Staphylococcus aureus*; Dx, diagnosis; WBC, white blood cells; LOS, length of stay; Abx, antibiotics; SE, staphylococcal enterotoxins.

<sup>c</sup> P values are for MRSA versus MSSA strains, except those marked with an asterisk, which are for PVL<sup>+</sup> versus PVL<sup>-</sup> MRSA strains.

*lukF-PV<sup>+</sup>* MSSA than in those with SSTIs caused by *lukF-PV*-negative MSSA (81% versus 57%, respectively). To assess the outcome of incision and drainage, selected predictors, including age, sex, presence of fever at diagnosis, methicillin resistance, and presence of the *lukF-PV* gene, were examined for an association (Table 2). There was a trend toward increased odds for incision and drainage among all patients with *lukF-PV<sup>+</sup>* infections (odds ratio [OR], 2.24; 95% confidence interval [95% CI], 0.79 to 6.35). When multivariable analysis was strat-

ified by status of methicillin resistance, the odds for incision and drainage among patients with *lukF-PV<sup>+</sup>* MSSA infections was significantly higher than among those with *lukF-PV*-negative MSSA infections (OR, 4.73; 95% CI, 1.14 to 19.68). In contrast, the presence of methicillin resistance was not associated with the outcome of incision and drainage (*P* = 0.7). In multivariable analysis, the presence of PVL remained a significant predictor for incision and drainage among MSSA strains.

Similarly to other studies, *lukF-PV<sup>+</sup>* MRSA strains were

TABLE 2. Multivariate analysis predicting need for incision and drainage

Characteristic	All strains (MRSA and MSSA)			MSSA strains only		
	P value	OR	95% CI	P value	OR	95% CI
Age	0.42	0.99	0.97–1.01	0.86	1.00	0.97–1.03
Male sex	0.05	2.57	0.98–6.70	0.06	3.44	0.95–12.50
Methicillin resistance	0.71	1.22	0.42–3.55			
PVL present	0.13	2.24	0.79–6.35	0.03	4.73	1.14–19.68
Fever present at Dx	0.016	5.67	1.39–23.13	0.07	5.50	0.89–33.92

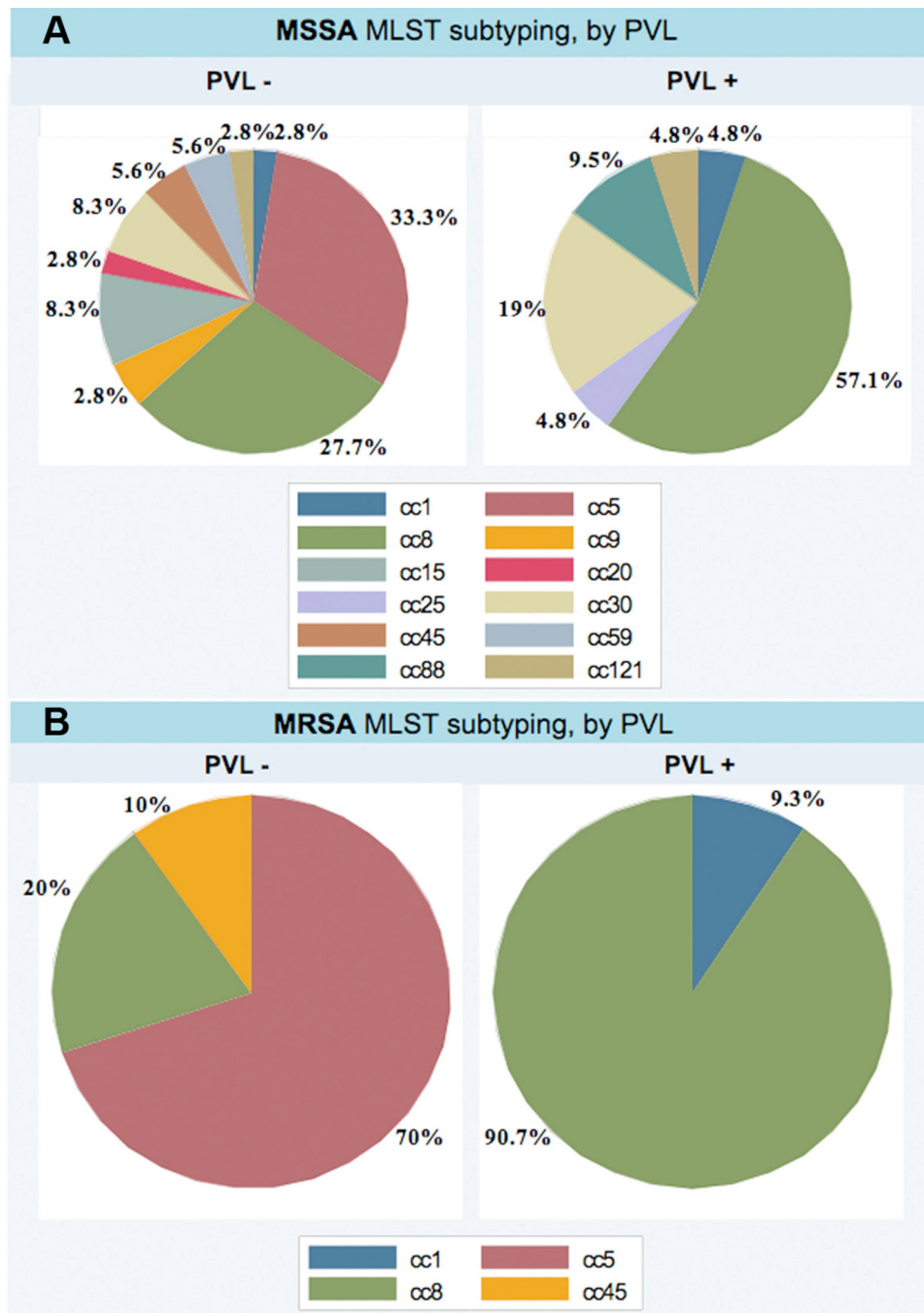


FIG. 1. Schemata of MLST of PVL-producing MSSA (A) and MRSA (B) strains.

mostly the epidemic strain t008/eGenomics type 1 (USA300). The three remaining were USA400 strains. Among 58 MSSA strains, 12 distinct MLST clonal types were identified. Pattern CC8 was most common, representing 57% of PVL-positive MSSA isolates, followed by CC30, CC88, CC25, CC121, and CC1, respectively (Fig. 1A). t008/eGenomics type 1 was identified in only 7 MSSA strains. In a New York prison population, this clone is also dominant among colonizing MSSA strains, of which 32.2% carry *lukF-PV* (4). Of note is that 4 of the t008/eGenomics type 1 strains (2 MRSA and 2 MSSA

strains) did not contain the *lukF-PV* gene. Similarly to USA300 strains, enterotoxin gene content was lower in *lukF-PV*<sup>+</sup> MSSA strains than in *lukF-PV*-negative MSSA strains (4 versus 5.7 genes, respectively;  $P = 0.002$  by  $t$  test). TSST, SEB, and SEC genes were absent in *lukF-PV*<sup>+</sup> MSSA and MRSA strains. The SEA gene was present in one MSSA strain from blood; however, all 3 USA400 strains had the SEC gene.

Polyclonality among *lukF-PV*<sup>+</sup> MSSA strains has been reported by several studies and stands in contrast to the clonal homogeneity among *lukF-PV*<sup>+</sup> MRSA strains (1, 5). This find-

ing could also be relevant for the pathogenesis of community-acquired MRSA (CA-MRSA) disease. First, most studies report that nasal colonization by MSSA is more common than that by MRSA. However, it is unknown how many colonizing MSSA strains produce PVL. Second, colonization with PVL-secreting MRSA or MSSA strains elicits a neutralizing polyclonal immune response to PVL, which is not protective (3) but is probably boosted by each infection. Third, it has been proposed, based on murine studies, that high titers of PVL neutralizing antibodies (Abs) inhibit clearance of MRSA infection (11). Finally, it is conceivable that recurrent infection with PVL-producing MRSA or MSSA strains may promote selection of strains that secrete higher levels of PVL, which is associated with worse SSTI in mice (8).

In summary, our data indicate that *lukF-PV*<sup>+</sup> MSSA strains, similarly to *lukF-PV*<sup>+</sup> MRSA strains, commonly cause complicated SSTIs in patients in the Bronx. The *lukF-PV* gene is uncommon in MSSA strains that cause septicemia without SSTI. As the epidemiology of CA-MRSA infection evolves, careful monitoring of emergence of genes encoding PVL in both MRSA and MSSA isolates is necessary to optimize the treatment and prevention of complicated SSTIs. This may further our understanding of *S. aureus* microevolution and how this process is affected by the human host response to its toxins.

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#### REFERENCES

1. **Chini, V., et al.** 2006. Spread of *Staphylococcus aureus* clinical isolates carrying Pantone-Valentine leukocidin genes during a 3-year period in Greece. *Clin. Microbiol. Infect.* **12**:29–34.
2. **Hamilton, S. M., et al.** 2007. In vitro production of Pantone-Valentine leukocidin among strains of methicillin-resistant *Staphylococcus aureus* causing diverse infections. *Clin. Infect. Dis.* **45**:1550–1558.
3. **Hermos, C. R., P. Yoong, and G. B. Pier.** 2010. High levels of antibody to Pantone-Valentine leukocidin are not associated with resistance to *Staphylococcus aureus*-associated skin and soft-tissue infection. *Clin. Infect. Dis.* **51**:1138–1146.
4. **Lowy, F. D., et al.** 2007. *Staphylococcus aureus* colonization and infection in New York State prisons. *J. Infect. Dis.* **196**:911–918.
5. **Monecke, S., P. Slickers, M. J. Ellington, A. M. Kearns, and R. Ehricht.** 2007. High diversity of Pantone-Valentine leukocidin-positive, methicillin-susceptible isolates of *Staphylococcus aureus* and implications for the evolution of community-associated methicillin-resistant *S. aureus*. *Clin. Microbiol. Infect.* **13**:1157–1164.
6. **Schlievert, P. M.** 2009. Cytolysins, superantigens, and pneumonia due to community-associated methicillin-resistant *Staphylococcus aureus*. *J. Infect. Dis.* **200**:676–678.
7. **Sinsimer, D., et al.** 2005. Use of a multiplex molecular beacon platform for rapid detection of methicillin and vancomycin resistance in *Staphylococcus aureus*. *J. Clin. Microbiol.* **43**:4585–4591.
8. **Varshney, A. K., et al.** 2010. Augmented production of Pantone-Valentine leukocidin toxin in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* is associated with worse outcome in a murine skin infection model. *J. Infect. Dis.* **201**:92–96.
9. **Varshney, A. K., et al.** 2009. Diverse enterotoxin gene profiles among clonal complexes of *Staphylococcus aureus* isolates from the Bronx, New York. *Appl. Environ. Microbiol.* **75**:6839–6849.
10. **Voyich, J. M., et al.** 2006. Is Pantone-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? *J. Infect. Dis.* **194**:1761–1770.
11. **Yoong, P., and G. B. Pier.** 2010. Antibody-mediated enhancement of community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Proc. Natl. Acad. Sci. U. S. A.* **107**:2241–2246.