Incidence, Risk Factors, and Outcomes of Panton-Valentine Leukocidin-Positive Methicillin-Susceptible Staphylococcus aureus Infections in Auckland, New Zealand

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Panton-Valentine leukocidin (PVL) has been linked to invasive community-acquired methicillin-resistant Staphylococcus aureus infections. However, the association between disease and PVL-positive methicillin-susceptible Staphylococcus aureus (MSSA) has not been widely reported. We aimed to examine the epidemiology of PVL in clinical MSSA isolates from patients presenting to Auckland City Hospital. Four hundred eleven MSSA clinical isolates and 93 nasal carriage isolates were collected and tested for the presence of the lukSF-PV genes using PCR. The results were examined in light of host and disease factors. Multilocus sequence typing (MLST) was performed on a random subset of isolates to ensure that there was no single PVL-positive MSSA clone responsible for disease in Auckland. The prevalence of the lukSF-PV genes in MSSA isolates associated with disease (124/335; 37%) was not significantly different from the prevalence of the lukSF-PV genes in MSSA nasal carriage isolates (29/93; 31% \( P = 0.33 \)). PVL-positive MSSA isolates in Auckland are genetically diverse and come from a number of different clonal complexes. PVL-positive infections peaked at between 10 and 20 years of age, with a subsequent decline. Pacific ethnicity, age, diagnosis of skin and soft tissue infection (SSTI), community-onset infection, and the need for surgical intervention were found by multivariate analysis to be independently associated with PVL-positive MSSA infection. More than one-third of MSSA infections in our patient population are caused by PVL-positive strains. Those patients with PVL-positive MSSA infection were more likely to be of Pacific ethnicity, be younger in age, have community-onset infection, have SSTI, and need surgical intervention.

Staphylococcus aureus is a nasal commensal that can be detected in up to 20 to 30% of the general population, one-third of whom are persistently colonized (28). S. aureus produces a wide variety of virulence factors that contribute to its ability to colonize, invade, and evade the immune system, which includes Panton-Valentine leukocidin (PVL), a bicomponent, pore-forming toxin encoded by two contiguous genes, lukF-PV and lukS-PV. PVL can cause either neutrophil lysis or apoptosis and contributes to tissue necrosis (25). PVL has been linked to skin and soft tissue infections (SSTIs), necrotizing pneumonia, and bone and joint infections in humans (3, 11, 17). Rabbit and skin and soft tissue infections (SSTIs), necrotizing pneumonia, and contributes to tissue necrosis (25). PVL has been linked to skin and soft tissue infections (SSTIs), necrotizing pneumonia, and bone and joint infections in humans (3, 11, 17). Rabbit and human leukocytes are highly sensitive to PVL-mediated leukocytosis (18), and animal studies have shown that PVL causes more severe disease in dermonecrosis (7, 27), osteomyelitis (6), and necrotizing pneumonia models (B. A. Diep, L. Chan, and P. Tattevin, presented at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009).

The presence of PVL has been extensively described for methicillin-resistant S. aureus (MRSA), specifically in association with staphylococcal cassette chromosome mec (SCCmec) type IV and also SCCmec type V (4, 25). The epidemiology of PVL-positive methicillin-susceptible S. aureus (MSSA) has not been reported as extensively, and the lukSF-PV genes are not exclusively linked to the presence of the SCCmec element. In the 1950s MSSA ST80 strains, which were associated with outbreaks of SSTI, harbored the lukSF-PV genes (22). There have also been recent reports of PVL-positive MSSA causing clusters of SSTI and necrotizing pneumonia (5, 15).

The vast majority of S. aureus strains in New Zealand are methicillin susceptible (MSSA); the prevalence of methicillin-resistant S. aureus (MRSA) remains low, at about 5% (12). New Zealand has a high incidence of S. aureus disease; the incidence of S. aureus bacteremia in the late 1990s was 41 cases per 100,000 adults per year (12). We aimed to examine the prevalence of the lukSF-PV genes in MSSA isolates responsible for disease and asymptomatic nasal carriage, to determine risk factors for infection with PVL-positive MSSA, and to examine the association between PVL and severity of disease.

MATERIALS AND METHODS

Clinical MSSA isolates. All MSSA isolates isolated from diagnostic specimens submitted to the Microbiology Laboratory of Auckland City Hospital from February to April 2008 were collected. These specimens were generally obtained at the request of the attending physician, midwife, or district nurse for clinical reasons. Duplicate isolates from the same patient were excluded. The isolates were stocked on nutrient agar (Difco Laboratories, Detroit, MI).
Nasal carriage isolates. MSSA isolates from nasal carriers were obtained from healthy population volunteers in the Auckland community during a separate study performed to examine the demographic features of nasal carriers in Auckland. These volunteers were recruited in public places spread across the Auckland region between February and November 2008; people with hospital contact in the previous 3 months were excluded.

Detection of the lukSF-PV genes. All isolates were cultured onto tryptic soy agar with 5% sheep blood (Difco Laboratories, Detroit, MI) and incubated aerobically overnight at 35°C. Nuclease acid was extracted from MSSA isolates as previously described (20). The PVL and nuc probes were synthesized with the nonfluorescent quencher BHQ1 (Biosearch Technologies, CA). Cal Fluor Gold 540 and FAM (6-carboxyfluorescein) were utilized as reporter dyes. Primers were synthesized based on oligonucleotide sequences described previously (20).

RESULTS

MSSA isolates were obtained from 411 patients for whom clinical details were available. The majority of the patients, 335/411 (81.5%), had a clearly identifiable focus of infection; 76/411 (18.5%) patients were not considered to have S. aureus infection and were excluded from analysis. We also obtained 93 MSSA isolates from nasal carriers for comparison. The prevalence of the lukSF-PV genes among the clinical MSSA isolates, 124/335 (37%), was not significantly different from the prevalence of the lukSF-PV genes among the nasal carriage isolates, 29/93 (31%) ($P = 0.33$). MLST confirmed that a diverse range of MSSA genotypes was PVL positive in Auckland: the 29 lukSF-PV-positive nasal carriage isolates belonged to eight clonal complexes (CC5, 9/29 [31%]; CC30, 7/29 [24%]; CC1, 6/29 [21%]; CC22, 2/29 [7%]; CC78, 2/29 [7%]; CC15, 1/29 [3%]; CC97, 1/29 [3%]; CC121, 1/29 [3%]), and the 24 randomly selected lukSF-PV-positive clinical isolates belonged to six clonal complexes (CC1, 12/24 isolates [50%]; CC121, 6/24 [25%]; CC30, 3/24 [13%]; CC5, 1/24 [4%]; CC8, 1/24 [4%]; CC78, 1/24 [4%]). Several predominant clonal complexes contained the majority of the lukSF-PV-positive MSSA isolates: 88% of the clinical isolates belonged to three predominant clonal complexes (CC1, CC30, and CC121), and 76% of the nasal carriage isolates belonged to three predominant clonal complexes (CC1, CC30, and CC121).

The prevalences of PVL-positive MSSA for patients within each infection group were 112/235 (48%) with skin and soft tissue infection, 4/13 (31%) with bone and joint disease, 2/11 (18%) with pneumonia, 2/36 (2%) with surgical-site infection, 1/13 (1%) with catheter-related infection, and 0/6 (0%) with primary bacteremia and endocarditis. Univariate analysis was performed to compare features of the PVL-positive MSSA infections ($n = 124$) to the features of the PVL-negative MSSA infections ($n = 211$) (Table 1). Histories of alcoholism and intravenous drug use were excluded from Table 1, as they were not routinely documented in clinical records. Factors associated with PVL-positive infections in the univariate analysis were investigated further by employing multivariate analysis. A diagnosis of SSTI, Pacific ethnicity, younger age, community-onset infection, and need for surgical intervention were all independently associated with the presence of a PVL-positive MSSA infection. The incidence of PVL-positive MSSA infections over the 2-month study period was 8.7 per 1,000 patients; it peaked for those individuals aged between 10 and 20 years, at 15.4 per 1,000 patients, and steadily decreased to 0.77 per 1,000 patients for those aged between 80 and 90 years ($r = -0.79; P = 0.01$). The incidence of PVL-negative infections showed no trend with age ($r = 0.20; P = 0.60$).

The majority of our MSSA infections, 232/335 (70%), presented as SSTIs, and univariate analysis of this subset was performed to directly compare features of PVL-positive MSSA
SSTIs to features of PVL-negative MSSA SSTIs. Of note, 112/232 (48%) of our patients presenting with MSSA SSTI had infection with a PVL-positive strain. Patients with PVL-positive MSSA SSTI were also 7.4 times as likely to require surgical drainage of cutaneous abscesses compared to patients with PVL-negative MSSA SSTI (95% confidence interval, 4.1 to 13.3).

### DISCUSSION

We found similar prevalences of *lukSF-PV* genes in both clinical and nasal carriage isolates of MSSA among a diverse genetic range of MSSA in Auckland. The prevalence of PVL-positive MSSA among clinical isolates of MSSA in Auckland,
37%, is similar to that reported in a study conducted in the Arkhangelsk region of Russia (26). However, other studies that used a methodology similar to that of our study reported much lower prevalence rates, ranging from 7 to 12% (13, 21, 29). In Auckland, PVL-positive MSSA was strongly associated with the diagnosis of SSTI; 48% of all MSSA isolates associated with SSTI were LukSF-PV positive. The reported incidence of PVL-positive MSSA SSTI in the literature ranges from 6.8% in a teaching hospital in Michigan (14) to 93% in a New York prison endemic with the PVL-positive USA300 MSSA strain (19).

The 2006 New Zealand Census revealed that the total population of Auckland consisted of the following ethnic groups: 61% European, 7% Maori, 11% Pacific, and 21% Asian (24). Compared to this census, Pacific and Maori patients are over-represented in the MSSA infection group, and European and Asian patients are underrepresented. PVL-positive MSSA infections were also more commonly associated with Pacific people than with people of other ethnic groups. Previous studies have also shown that Maori and Pacific people living in New Zealand are at a higher risk of disease caused by S. aureus (12), and socioeconomic deprivation is likely to play an important role in the development of infectious disease in Auckland (2).

We found that people with MSSA infection were more likely to live in areas with higher NZDep2006 scores (more deprived) than the general population, but there was no significant association between the prevalence of PVL-positive MSSA infection and increasing NZDep2006 scores.

In this study, we compared the differences in severities of PVL-positive versus PVL-negative infections. We found that patients with PVL-positive MSSA infection were 3.9 times more likely to require surgery than those with PVL-negative MSSA infection. Within the SSTI group, this distinction was even more pronounced: those with PVL-positive MSSA SSTI were 7.4 times more likely to require surgery than those with PVL-negative MSSA SSTI. However, there was no significant difference between the two groups in terms of other measures of disease severity: the number of surgical procedures required, rate of bloodstream invasion, duration of admission, 30-day readmission rate, and crude 30-day mortality rate.

In conclusion, we found that more than one-third of MSSA infections in our region were caused by PVL-positive strains. PVL-positive MSSA infection was strongly associated with patients of Pacific ethnicity, younger age, diagnosis of SSTI, community-onset infection, and need for surgical intervention. This study showed a peak in PVL-positive infections in childhood and young adulthood, with a decline in later years. Further studies are required to elucidate the underlying reasons for this finding.

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