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ORIGINAL ARTICLE BACTERIOLOGY

Panton–Valentine leukocidin associated staphylococcal disease: a cross-sectional study at a London hospital, England

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Abstract

Recently, there has been international concern at the rapid emergence of highly pathogenic strains of *Staphylococcus aureus* associated with a toxin called Panton–Valentine leukocidin (PVL). In the UK, these strains are considered to be rare and mainly severe. We estimate the proportion of staphylococcal infections that are caused by strains containing the PVL genes, and describe risk factors for these infections. Three hundred and ninety consecutive *S. aureus* clinical isolates, submitted for routine diagnostic purposes were screened for PVL genes. Risk factors for infection were identified from the patient medical record. 9.7% (95% CI 7.0–13.1%) of clinical isolates and 20.8% of skin and soft tissue specimens contained the genes for PVL. Methicillin-resistant *S. aureus* with PVL was rare (0.8% of all isolates) but PVL with methicillin-sensitive *S. aureus* was common (9.0% of all specimens). PVL infection was more frequent in males (OR 3.0, 95% CI 1.3–7.0), and in young adults aged 20–39 years (OR 3.7, 95% CI 1.3–10.4). Over half of PVL positive *S. aureus* infections originated in patients based in the community. Community-onset PVL-associated disease is common in the UK and mainly causes skin and soft tissue infections that do not require admission to hospital. Consideration should be given to current infection control strategy, which advocates household contact screening and decolonization on the assumption that PVL-associated disease is rare.

Keywords: Community-acquired infection, epidemiology, infection control, risk factors, *Staphylococcus aureus*
Original Submission: 12 October 2009; **Revised Submission:** 3 December 2009; **Accepted:** 14 December 2009
Editor: G. Lina
Article published online: 23 December 2009
Clin Microbiol Infect 2010; **16:** 1644–1648
10.1111/j.1469-0691.2010.03153.x

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Introduction

Panton–Valentine leukocidin (PVL) containing strains of *Staphylococcus aureus* have emerged worldwide as an important cause of severe skin and soft tissue infections [1], necrotizing pneumonia and sepsis [2,3]. These strains mainly affect young and healthy individuals who have not had contact with healthcare, suggesting that infection is acquired in the community [4,5]. Cases of PVL containing *S. aureus* (PVL-SA) pneumonia have been reported across Europe [2,6,7] but the prevalence of PVL strains amongst isolates submitted to national reference units is variable [1,8–11].

PVL is a cytotoxin that causes leukocyte destruction and tissue necrosis [12]. Although there is agreement that PVL strains are epidemiologically linked to severe skin infections and necrotizing pneumonia [1,2], conflict remains over whether PVL is itself pathogenic [13–15]. Outbreaks of PVL-SA associated disease have been described in diverse populations such as the military, prisoners, Alaskan natives and sports-teams [16–19] and persistent close contact is a key factor in transmission [20]. Infection control measures focus on good hygiene, avoiding sharing personal items and environmental cleaning.

PVL-SA strains are considered to be rare in the UK [21]. In 2002, <2% of a sample of *S. aureus* isolates referred to the UK Staphylococcal Reference Unit contained the PVL genes [11]. This is likely to under-estimate the true burden of disease in the community because reference units only receive a small number of highly selected isolates that are unrepresentative of the whole spectrum of clinical disease. Because PVL-SA surveillance is not systematic and current UK national staphylococcal surveillance depends largely on specimens from bacteraemias, an increase in PVL containing strains in the community could easily pass undetected.

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We estimate the proportion of staphylococcal infections that are caused by PVL containing strains and describe risk factors for these infections.

Materials and Methods

Study design and setting

This prospective cross-sectional study was conducted at the Royal Free Hampstead NHS Trust, a large teaching hospital in North London which has approximately 900 beds and receives approximately 700 000 patients per year. Locally, approximately 250 000 people use the general health services at the Trust (0.4% of the total UK population), whereas patients using specialized services travel from all over the country and abroad. The Microbiology laboratory receives in the region of 400 000 clinical specimens from inpatients, outpatients and general practice. Between 15 July and 11 September 2007 *S. aureus* isolates were collected from hospital and community specimens and investigated for the presence of the PVL genes using real-time PCR. Data were anonymized by removing all patient-identifying details and allocating a unique study code to each specimen, linked to the Patient Administration System (PAS) database. Information on hospital admission between 1 January 2006 and 11 September 2007 was obtained from the PAS database for each patient. Ethical approval was obtained from the Royal Free Hospital and Medical School Local Research Ethics Committee (reference 07/H0720/146).

Inclusion and exclusion criteria

Three hundred and ninety consecutive, unselected *S. aureus* isolates were collected from hospital and community specimens and were stored. Subsequent isolates from the same patient and methicillin-resistant *S. aureus* (MRSA) screening specimens were excluded.

Bacterial isolates

Identification and antimicrobial susceptibility testing of *S. aureus* isolates were performed using the Phoenix system (Becton Dickinson, Oxford, UK). All isolates were stored on cryogenic beads at –70°C until PCR was performed.

DNA extraction and real-time PCR of PVL genes

Isolates were inoculated from beads onto blood agar and incubated at 35°C overnight. DNA was extracted from the cultured isolates using an achromopeptidase lysis procedure [22]. Real-time PCR was used to detect a 138-bp region spanning the *lukS* and *lukF* genes and all positive results were confirmed by sequence analysis. The original PCR target described by Lina *et al.* [1] was shortened from 433 bp for use on a real-time platform (Wallis M & Steer J, Plymouth Hospitals NHS Trust, personal communication). Real-time PCR was performed on a Rotor-Gene 3000 (Qiagen, Crawley, UK) in a final volume of 25 μl containing 1 $\times$ TaqMan Universal PCR Master Mix (Applied Biosystems, Warrington, UK), 0.9 μM of each primer (PVLF: 5′-ATG TCC TTT CAC TTT AAT TTC ATG AGT TTT-3′; PVLR: 5′-CAT GCT ACT AGA AGA ACA ACA CAC TAT GG-3′) and 0.2 μM probe (–6 FAM AAA TGC GTT GTG TAT TCT AGA-MGB). PCR cycling conditions were; one hold at 50°C for 2 min followed by a second hold at 95°C for 10 min followed by 35 cycles of 95°C for 15 s and 60°C for 60 s. PCR testing was performed by a researcher who was blind to the exposure status of each bacterial sample.

Study measurements

Clinical profile. Data were extracted on infection type from the clinical information on the specimen request form. Specimens from abscesses, impetigo, cellulitis, folliculitis, carbuncles and paronychias were grouped as skin and soft tissue infections and analyzed separately from wound infections. The source was defined as the location of the patient when the sample was taken and specimens from all sources except hospital inpatients were classified as community-onset infections.

Risk factors for infection. Data were extracted on patient age, gender, previous hospitalizations and history of *S. aureus* infection or colonization. Hospital admission was recorded as the total number and total duration of admission in days. Outpatient attendance was defined as total number of outpatient appointments during the same period. Data on age, hospital admission and outpatient attendance were included as categorical variables reflecting the underlying distribution of the data.

Statistical analysis

A sample size of 400 isolates was required to estimate a PVL prevalence of 10% with a 95% CI of 7–14%. We used STATA, version 10 (StataCorp, College Station, TX, USA), for all data management and analyses. Descriptive statistics were used to summarize the clinical and demographic characteristics of the patients and the microbiological characteristics of the sample. We estimated odds ratios for the association between age, gender and PVL-SA disease and examined the effect of healthcare contact, infection type, infection source and previous isolation of *S. aureus*. A logistic regression model was developed to identify risk factors for PVL disease by sequentially adding in factors associated with outcome,

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starting with those most strongly associated, and assessing for interaction with age. Results were presented as adjusted ORs with 95% CI and p-values.

Results

Three hundred and ninety samples were available for analysis. Thirty-eight of 390 specimens (9.7%, 95% CI 7.0–13.1%) contained the genes for PVL, and 66/390 (16.9%, 95% CI, 11.8–18.6%) were methicillin resistant. 0.8% (3/390) of *S. aureus* isolates contained PVL genes and were methicillin resistant and 9.0% (35/390) contained PVL and were methicillin-sensitive.

Clinical and demographic characteristics of PVL-SA positive cases

20.8% (22/106) of isolates from skin and soft tissue infections and 10% (1/10) from pus contained the PVL genes (Table 1). PVL-SA strains were rare amongst infections that are traditionally healthcare-associated such as ulcers (0/25). Compared to individuals aged <20 years, the odds of PVL-SA-related infection were highest in subjects aged 20–39 years, and reduced thereafter with increasing age (OR 3.2, 95% CI 1.1–8.7, p 0.02 (test for trend). Men had approximately twice the odds compared to women of having PVL-SA-related disease, [13.4% (29/216) vs. 5.3% (9/170), OR 2.8, 95% CI 1.3–6.1, p 0.008]. Compared to infections caused by PVL-SA negative strains, a higher proportion of PVL-SA infections had their onset in the community [63.9% (225/352) vs. 71.1% (27/38)].

PVL-SA infection was strongly associated with skin and soft tissue disease and these samples were four-fold more likely to contain the PVL genes compared to all other sample types [20.8% (22/106) vs. 5.6% (16/284), OR 4.4, 95% CI 2.2–8.9, p <0.001]. The odds of PVL-SA disease were independent of whether an isolate was methicillin-resistant and whether the patients had a previous *S. aureus* infection.

Prior hospital contact and risk of PVL-SA-related disease

Patients who had not attended outpatients or been admitted to hospital were at increased risk of PVL-SA infection. Compared to patients who had spent at least 3 days in hospital in the previous 18 months, patients who had not been admitted were four-fold more likely to be infected with a PVL positive strain [12.4% (26/209) vs. 3.2% (3/95), OR 4.4, 95% CI 1.3–15.0] (Table 2). The odds of PVL infection were associated with both the number of previous admissions and the duration of previous admissions. Compared to individuals who frequently attended outpatients (more than five appointments in the last 18 months), patients who had not had an outpatient appointment were more than three-fold as likely to be infected with a PVL positive strain [11.7% (24/206) vs. 3.2% (3/94), OR 4.0, 95% CI 1.2–13.8].

In a multivariate logistic regression model, male gender and skin and soft tissue disease were all independently associated with PVL when adjusted for all other variables in the model (p <0.05). There was no interaction with age for any of the variables included in the multivariate analysis. Inclusion of methicillin susceptibility data [methicillin-sensitive *S. aureus* (MSSA) or MRSA] did not improve the model (p 0.7) and a separate analysis stratified by *S. aureus* type identified the same risk factors for PVL disease as in the combined analysis.

Discussion

PVL associated with MRSA was rare in the present study, but PVL associated with MSSA was common and almost

TABLE 1. Proportion of Panton–Valentine leukocidin (PVL) positive and patient age by specimen type

Sample type[a]	*n* (%)	Median age in years (interquartile range)	PVL-SA+/Total SA (%)
Skin/soft tissue infections	106 (27.2)	33.2 (17.1–58.3)	22/106 (20.8)
Wounds	120 (30.8)	60.9 (39.8–78.7)	10/120 (8.3)
Bacteraemias	3 (0.8)	73.6 (52.0–83.9)	0/3 (0)
Indwelling device	29 (7.4)	60.8 (47.6–67.3)	0/29 (0)
Osteomyelitis	3 (0.8)	54.1 (51.4–54.1)	0/3 (0)
Pus	10 (2.5)	61.6 (41.1–85.9)	1/10 (10.0)
Surface swabs[b]	62 (15.9)	50.8 (5.6–69.7)	2/62 (3.2)
Ulcers	25 (6.4)	68.3 (51.7–80.2)	0/25 (0)
Other	20 (5.1)	51.6 (39.7–72.7)	2/20 (10.0)
Respiratory	2 (0.5)	55.7 (13.9–97.5)	0/2 (10.0)
Unknown	10 (2.6)	24.3 (2.0–40.6)	1/10 (10.0)
Total	390 (100.0)	51.4 (28.4–72.9)	38/390 (9.7)

[a]Sample type recorded directly from hospital microbiology database.
[b]Samples taken from the skin surface not labelled as skin and soft tissue infections, wounds or ulcers.
SA, *Staphylococcus aureus*.

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TABLE 2. Risk of PVL-SA infection associated with hospital inpatient admission or outpatient attendance in the previous 18 months

Patient characteristics	PVL-SA positive (n = 49)	OR (95% CI)	p-value
Medical history			
Outpatient/inpatient in the last 18 months	18/220 (8.2%)	1.0	0.2
None	20/170 (11.8%)	1.5 (0.8–2.9)	
Number of admissions to hospital			
2 or more	3/95 (3.2%)	1.0	0.01[a]
1	9/86 (10.4%)	3.6 (0.9–14.0)	
None	26/209 (12.4%)	4.4 (1.3–15.0)	
Duration of admissions/days			
>3	5/120 (4.2%)	1.0	0.02[a]
1–3	7/61 (11.5%)	3.0 (0.9–10.0)	
None	26/209 (12.4%)	3.3 (1.2–8.4)	
Number of outpatient appointments			
6+	3/94 (3.2%)	1.0	0.04[a]
1–5	11/90 (12.2%)	4.2 (1.1–16.0)	
None	24/206 (11.7%)	4.0 (1.2–13.8)	

[a]Test for trend.
PVL-SA, Panton–Valentine leukocidin containing *Staphylococcus aureus*.

three-quarters of PVL-SA infections occurred in patients based in the community.

Strengths and limitations of the study

The present study estimates the proportion of sampled staphylococcal infections that contain the PVL genes and is the first in the UK to use an unselected sample of clinical isolates.

Although we accurately measured the prevalence of PVL amongst sampled infections, selection bias is likely because not all infection types have an equal probability of being sampled. Most patients with skin and soft tissue infections do not have specimens taken for microbiological diagnosis. This will tend to under-represent the total amount of skin and soft tissue disease, and may under-estimate the overall prevalence of PVL-SA infections. Conversely, PVL production may be up-regulated with low-dose flucloxacillin, tending to over-estimate the prevalence of PVL-SA amongst infections treated with β-lactam antibiotics in the community [23].

The present study was limited by the quality and type of data available in the patient hospital record. We could not account for admissions or outpatient attendance at hospitals other than the Royal Free Hampstead NHS Trust, and we did not classify infections in hospitalized patients in the first 48 h of admission as community-onset. These assumptions may have respectively over and under-estimated the importance of community onset infections. We had incomplete data on the patients' past medical history, and no information on antibiotic usage or behavioural risk factors. Because we could not adjust for these factors in our analysis, they may have confounded the associations we report. Data were limited on the provenance of samples classified as surface swabs, other and unknown origin, and PVL-SA prevalence varied widely between these categories. Surface swabs are mainly taken from superficial skin lesions, whereas most specimens classified as 'other' were obtained from individuals with a wide range of systemic illnesses. This may explain the heterogeneity in proportion of infections caused by strains containing the PVL genes.

Our analysis suggests that MSSA with PVL genes are common and mainly cause skin and soft tissue infections that do not require hospital admission. Our findings are likely to be generalizable to other hospitals, suggesting that surveillance data significantly under-estimate the burden of PVL-SA infection. This has implications for UK infection control policy, which advocates screening and/or treatment of PVL-SA cases and their contacts [21] on the assumption that cases are rare and serious. By contrast, if PVL cases are common and usually not severe, widespread screening and decolonization will be expensive, impractical and may expose many individuals to unnecessary antibiotic treatment.

PVL-associated disease was commoner in males and we speculate that gender-specific physical factors, such as increased body hair, might predispose to infection. We lack data on individual patient outcomes, but almost half of PVL strains were isolated from patients attending outpatients or general practice, suggesting that they were sufficiently well to receive treatment in the community. Because skin and soft tissue infections are one of the commonest clinical presentations in general practice [24], we speculate that PVL containing strains are responsible for a large number of non-severe skin and soft tissue infections across the UK that are managed effectively without hospital treatment.

The number of PVL-SA cases reported to the staphylococcal reference unit has doubled between 2005 and 2006 [21], which may represent improved case ascertainment or a real increase in the number of cases. A generalized increase in PVL-SA prevalence might be expected to lead to an increase in severe disease, and this is supported by data from hospital episode statistics: admissions for severe

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staphylococcal disease such as pneumonia and staphylococcal scalded skin syndrome have increased five-fold between 1989 and 2004 [25].

Policy in this area must be informed by improved surveillance to accurately determine the prevalence of PVL-SA infection, coupled with an understanding of the contribution of PVL and other toxins and virulence factors to disease manifestation and severity. Until we identify patient-level risk factors for severe disease, a universal policy is impractical. We propose that guidance on decolonization and screening of PVL-SA cases and contacts should be based on clinical presentation and severity, and not simply on the presence of the PVL gene.

Acknowledgements

We thank Professor Stephen Gillespie, Regional Microbiologist, for technical advice and laboratory funding and Dr Angela Kearns at the Health Protection Agency.

Transparency Declaration

This work was supported by an in-house grant from the Royal Free Hampstead NHS Trust. L.S. is funded by a Medical Research Council Training Fellowship (G0501879). Funding sources did not play any role in the design or analysis of this study. All authors declare that they have no commercial or other association that might pose a conflict of interest.

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