

Prevalence of Panton Valentine Leukocidin in Carriage and Infective Strains of *Staphylococcus aureus* at a Referral Hospital in Kenya

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ABSTRACT

Panton valentine leukocidin (PVL) is a pore forming exotoxin that is expressed by some *Staphylococcus aureus* (*S. aureus*) strains and is thought to add to its virulence. The prevalence of PVL in carriage and disease causing strains varies considerably from region to region. This study compared the prevalence of the PVL gene in *S. aureus* isolates obtained from healthcare workers and from patients seen at the Aga Khan University Hospital Nairobi (AKUHN). *S. aureus* isolates obtained from healthcare workers and patients attended to at AKUHN between July 2010 and March 2011 were used for this study. Forty five *S. aureus* isolates from healthcare workers and 63 from clinical specimens obtained from 59 patients were analysed for the PVL gene. The prevalence of PVL in isolates from healthcare workers was 24.4% compared to 39.7% in the isolates causing infection ($P = 0.098$). PVL prevalence was 58.8% in *S. aureus* isolates obtained from skin and soft tissue infections (SSIs) compared to 25.0% in carriage isolates ($P = 0.002$, OR 4.29). Prevalence in isolates from invasive infections was 11.1%. Patients with PVL positive *S. aureus* were younger than those with PVL negative isolates ($P = 0.082$). The high prevalence of PVL is comparable with that reported in other African countries. The significance of the high prevalence of PVL in *S. aureus* isolates carried by health care workers at AKUHN is unclear at the moment. PVL prevalence is significantly higher in *S. aureus* isolates causing SSIs compared to carriage and invasive isolates.

Keywords: *Staphylococcus aureus*; *S. aureus*; Panton Valentine Leukocidin; PVL

1. Introduction

Panton valentine leukocidin (PVL) is a pore forming exotoxin that is expressed by some *Staphylococcus aureus* (*S. aureus*) strains. It was named after Sir Philip Noel Panton and Francis Valentine when they associated it with soft tissue infections in 1932 [1]. It is composed of two water soluble proteins LukF-PV and LukS-PV that specifically lyse polymorphonuclear cells and monocytes by creating pores in their cell membranes [2].

S. aureus strains producing PVL have been associated with a variety of illnesses ranging from skin and soft tissue infections to serious infections like necrotizing pneumonia that are invariably fatal [3]. Unfortunately, the necrotizing pneumonia caused by these strains is more common in young healthy adults and mortality is usually very high despite treatment [4]. Studies carried out on rabbit models have shown that the increased lung

damage caused by PVL positive strains is mediated by toxic granules released by polymorphonuclear cells due to PVL mediated cytolysis. However, lung damage can also occur in the absence of PVL but the morbidity is less [5].

The prevalence of PVL in *S. aureus* isolates obtained from clinical specimens has been found to vary significantly from country to country with prevalence as high as 57% seen in isolates from west African countries compared to 9.7% in England [6,7]. Whether the presence of PVL translates to more severe disease in humans is debatable however they are associated with recurrent soft tissue infections. An observational study carried out in New Zealand found that patients with PVL-positive methicillin susceptible *S. aureus* (MSSA) Surgical Site Infections were 7.4 times more likely to require surgical drainage of cutaneous abscesses compared to patients with PVL negative infections [8]. However, some experimental and epidemiologic studies have shown that PVL may not be a major virulence factor in causing

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community acquired methicillin resistant *S. aureus* infections (MRSA) [9,10]. Bae *et al.* showed that factors other than the presence of PVL are the primary determinants of clinical outcome in complicated skin and skin structure infections caused by MRSA [11]. Some of the *in-vitro* studies that have questioned the role of PVL in pathogenesis were based on murine models whose neutrophils have been shown to be inherently resistant to PVL. PVL has been shown to act differentially on neutrophils from various animal species and as such careful selection of animal models is required to aid in properly defining the pathological role of PVL. In this regard, rabbit models have been found to be the best human surrogates [12, 13].

Few studies have attempted to compare the prevalence of PVL in colonizing and disease causing strains of *S. aureus* [14-16]. We hypothesized that the prevalence of PVL would be higher in *S. aureus* isolates causing infection compared to carriage isolates given the evidence of increased virulence conferred by PVL [8,17]. This study compared the prevalence of the PVL gene in *S. aureus* isolates obtained from the anterior nares of healthcare workers and *S. aureus* isolates from patients seen at the Aga Khan University Hospital Nairobi (AKUHN).

2. Methodology

AKUHN is a 254-bed private referral hospital with satellite clinics and laboratories serving a sizeable population in Nairobi and other major towns in Kenya. The hospital has all major medical disciplines including a state of the art heart and cancer centre, dialysis units, adult and paediatric high dependency and intensive care units. The laboratory at the main hospital has Microbiology, Haematology, Clinical chemistry and Anatomical pathology sections that are all ISO 15189:2007 accredited by the South African National Accreditation System (SANAS).

2.1. Samples

S. aureus isolates obtained from healthcare workers and patients attended to at AKUHN were used for the study. The isolates from healthcare workers were obtained as part of a previous study looking at nasal carriage of MRSA at AKUHN between July and December 2010. Two hundred and sixty one healthcare workers were selected at random from all clinical departments using a predefined procedure. The departments included pathology, radiology, accident and emergency, paediatrics, surgery, obstetrics and gynaecology, adult medical wards, intensive care and high dependency units, physiotherapy and operating theatres. Participation was voluntary and written informed consent was obtained [18]. *S. aureus* isolates from patients were obtained sequentially from clinical specimens submitted to the Microbiology divi-

sion of the Pathology department for routine diagnostic and surveillance cultures between January and March 2011. These included blood, urine, pus swabs, surveillance swabs (nasal, axillary and groin) and body fluids. Bacterial isolates were frozen in brain heart infusion with 10% glycerol at -80°C until analysis.

2.2. Identification of *S. aureus*, *MecA* and PVL Genes

Frozen isolates were thawed at room temperature and cultured on to blood agar plates (Oxoid Ltd., Basingstoke, UK). Colonies morphologically resembling *S. aureus* were identified using standard bench methods which included gram stain, catalase test, tube and slide coagulase tests. Confirmed *S. aureus* strains were subjected to disc diffusion using 1 μg oxacillin and 30 μg cefoxitin discs (BD BBL, MD, USA) to identify MRSA. Interpretation of the tests was based on Clinical Laboratory Standards Institute guidelines [19]. Genomic DNA was extracted from all the *S. aureus* isolates using QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. All *S. aureus* isolates were then tested for the presence of *MecA* and PVL genes using the Genotype MRSA kit version 2.0 (Hain Lifescience, Nehren, Germany). Appropriate controls were included for all tests.

2.3. Ethical Approval

Ethical approval was obtained from the AKUHN scientific and ethics committees.

2.4. Statistics

Descriptive statistics were presented as means or proportions as appropriate. The Kolmogorov Smirnov test was used to test for normality of data. All inferential statistics were exploratory. The independent sample t-test was used to compare means for parametric data while the Mann Whitney U test was used for non-parametric data. Chi-square test was used to compare proportions. All tests were two sided. A p value less than 0.05 was considered statistically significant. Statistical analysis was carried out using SPSS version 15.0 (SPSS, Inc., Chicago IL).

3. Results

Forty five *S. aureus* isolates from healthcare workers and 63 from clinical specimens obtained from 59 patients were analysed for the presence of the PVL gene. The mean (SD) age in years for the healthcare workers and patients was 34.5 (6.7) and 34.3 (22.1) respectively. The difference was not statistically significant ($t(71.6) = 0.078$, $P = 0.938$). There were 27 (60%) and 37 (62.7%) males among healthcare workers and patients respec-

tively.

The prevalence of *PVL* in the isolates obtained from healthcare workers was 24.4% (11/45) compared to 39.7% (25/63) in the isolates obtained from patients ($P = 0.098$). The mean age for patients whose specimens had *PVL* positive *S. aureus* was significantly lower than those with *PVL* negative *S. aureus* with mean (SD) ages in years of 25.7 (17.1) and 42.4 (23.2) respectively ($U = 271$, $P = 0.004$). Amongst health care workers, a similar difference in age was not seen between carriers of *PVL* positive and *PVL* negative *S. aureus*. Their mean ages (SD) in years were 35 (6.5) and 34.4 (6.9) respectively ($U = 163.5$, $P = 0.540$).

The prevalence of *PVL* in the different specimen types is shown in **Table 1**. Pus swabs had the highest prevalence of 57.1% (20/35) and of the 10 blood specimens, only 1 had *PVL*. The prevalence of *PVL* in carriage strains obtained from both healthcare workers and patients was 25.0% compared to 42.3% (22/52) in disease causing strains. This difference wasn't statistically significant ($P = 0.059$, OR 2.2; 95% CI: 0.97 - 4.98). However, a comparison between *PVL* prevalence in *S. aureus* isolates from SSIs and those from non SSIs (4 urine, 10 blood, 2 ascitic fluid, 1 pus swab and 1 sputum) showed a significant difference with the prevalence being higher in SSIs ($P = 0.003$, OR 11.43; 95% CI: 2.26 - 57.8). A similar comparison between SSI isolates and screening swabs (45 nasal swabs from healthcare workers and 11 surveillance swabs from patients) also showed a significant difference with a higher prevalence in SSIs ($P = 0.002$, OR 4.29; 95% CI: 1.72 - 10.67).

Table 2 shows the prevalence of *PVL* in *S. aureus* isolates from SSIs, non SSIs and screening swabs. The mean (SD) age for patients with SSIs was 30.4 (22) years while that for patients with invasive infections was

Table 1. Prevalence of *PVL* gene in *S. aureus* isolates obtained from different patient specimens.

Specimen type	<i>PVL</i> gene number (percentage)		Total
	Absent	Present	
Urine	4 (100%)	0 (0%)	4
Pus swab	15 (42.9%)	20 (57.1%)	35
Blood	9 (90%)	1 (10%)	10
Surveillance swab ^a	8 (72.7%)	3 (27.3%)	11
Ascitic fluid	1 (50%)	1 (50%)	2
Sputum	1 (100%)	0 (0%)	1
Total	38 (60.3%)	25 (39.7%)	63

^aSurveillance swabs include nasal, axillary and groin swabs collected from patients as part of screening on admission into the intensive care and high dependency units.

Table 2. Prevalence of *PVL* in colonizing and disease causing strains of *S. aureus*.

Specimen type	<i>PVL</i> gene: number (percentage)		Total
	Absent	Present	
Skin and soft tissue ^a	14 (41.2%)	20 (58.8%)	34
Non skin and soft tissue ^b	16 (88.2%)	2 (11.1%)	18
Screening swab ^c	42 (75.0%)	14 (25.0%)	56
Total	74 (63.8%)	42 (36.2%)	108

^aIncludes all pus swabs obtained from skin and soft tissue infections (SSIs);

^bIncludes urine, blood, sputum, pus and ascitic fluid representing invasive infections; ^cIncludes nasal swabs from healthcare workers and surveillance swabs (nasal, axillary and groin) from patients.

41.2 (24.3) years. This difference wasn't statistically significant ($U = 208.5$, $P = 0.082$).

Only 9 of the 63 (14.3%) *S. aureus* isolates obtained from the patient specimens had the *MecA* gene detected. Of these, 3 also had the *PVL* gene. Two of the isolates were from pus swabs and the other was from ascitic fluid.

4. Discussion

The precise role of single virulence factors in the causation of infectious disease is hard to establish and *PVL* is no exception. Several studies do suggest that *PVL* is one of the major virulence factors contributing to the morbidity and mortality attributed to *S. aureus* [3,4,17]. This contribution has however been put to question by studies that have shown a better clinical outcome for patients with SSIs caused by *PVL* producing MRSA compared to strains not producing *PVL* [11,20]. Otto *et al.* have suggested that factors other than the mere presence of *PVL* are the primary determinants of clinical outcome in patients with *S. aureus* SSIs [21]. This study found the prevalence of *PVL* in *S. aureus* isolates causing infection to be 42.3%. Compared to the prevalence in carriage strains, the difference wasn't statistically significant but tended towards significance with a P value of 0.059. Feil *et al.* showed clonal similarity between carrier and disease causing strains of *S. aureus* from a localized population in Oxfordshire, United Kingdom suggesting that *S. aureus* infections are opportunistic in nature and as such cases of disease are thought to represent a random sample of the much larger reservoir of carriage strains. No significant differences should therefore exist between carriage and disease causing strains of *S. aureus* [22]. In this study, a comparison between the prevalence of the *PVL* gene in *S. aureus* isolates from patients with SSIs and those with non SSIs showed a significantly higher prevalence in the former. This finding is in keeping with what has been previously documented. Prevost *et al.*

found that 23.6% of *S. aureus* isolates from cutaneous infections had the *PVL* gene compared to 1.4% of blood isolates [23]. A similar finding was seen in Gabon where the prevalence of *PVL* in isolates from abscesses was 100% while in blood isolates it was 54.5%. Despite similarities in clonality as determined by multilocus sequence typing (MLST) between carriage and disease causing strains of *S. aureus*, true differences may actually exist. The apparent clonal similarity between carriage and invasive *S. aureus* strains as reported by Feil *et al.* may be as a result of limitations of the MLST typing method used which only looks at housekeeping genes [24]. It is therefore possible that there could be differences in the population structure of *S. aureus* based on variations in accessory genes that MLST could miss. The differential occurrence of the *PVL* gene in *S. aureus* isolates causing SSIs and non SSIs in this study may be a reflection of such variability. *PVL* producing *S. aureus* appear to be successful pathogens with a preference for SSIs as seen in this study.

Nasal carriage of *S. aureus* by healthcare workers at AKUHN has previously been reported to be 18.3% while MRSA was found to be absent [18]. The isolates obtained during the carriage study were used in this study to determine *PVL* gene prevalence in carriage strains of *S. aureus* which was found to be 24.4%. This is comparable with prevalence found in other African countries which ranges between 17% and 74% but is considerably higher than that found in other continents where the prevalence of *PVL* in carriage isolates was found to range from 0% to 10.6% [6,14-16,25-27]. It is possible that there is a *S. aureus* clone harboring the *PVL* gene circulating amongst healthcare workers at AKUHN hence the high prevalence. However, *PVL* is commonly associated with community acquired isolates and the high prevalence could indeed be a true reflection of the *status quo* as seen in the other African studies [28]. In Mali, a pandemic clone (ST 152) that comprised 23.9% of carriage isolates was identified and all the isolates belonging to this clone were found to harbour the *PVL* gene [29]. The carriage isolates in the Mali study were obtained from patients admitted for emergency surgery and most likely represent community isolates. The prevalence of *PVL* in *S. aureus* isolates obtained from a healthy non hospital based population in the community served by AKUHN may help in better understanding the epidemiology of these isolates. The significance of the high prevalence of *PVL* in *S. aureus* isolates carried by healthcare workers at AKUHN is unclear at the moment. Comparative genomics of the carriage and disease causing strains may help ascertain the disease causing potential of the carriage strains by determining clonality.

Only 9 *S. aureus* isolates from clinical specimens were found to possess the *MecA* gene and of these 3 had the

PVL gene. These 3 isolates may be community acquired MRSA given that *PVL* presence is more common in community acquired isolates [30]. There is a paucity of published data on community acquired MRSA (CA-MRSA) isolates harbouring the *PVL* gene from East Africa. The emergence and spread of such isolates has already been reported in Algeria, Cameroon, Morocco, Nigeria, and Senegal [6,31,32]. Globally, CA-MRSA has been the source of numerous outbreaks causing significant morbidity and mortality [33-35]. Fortunately, none of the carriage *S. aureus* isolates from health care workers at AKUHN harboured both the *MecA* and *PVL* genes. However, such strains could still make their way from the community to the hospital like the USA 300 strain, a *PVL* producing CA-MRSA, which has begun to displace traditional hospital acquired MRSA strains in the United States [34,36]. Continuous monitoring of microbiological data on both community and hospital acquired infections and appropriate containment measures will go a long way to ensure that *PVL* producing CA-MRSA doesn't become endemic at AKUHN.

This study found that patients with *PVL* positive *S. aureus* were younger than patients with *PVL* negative strains. This finding is not unique to this study. Tong *et al* analysed data from two large, multinational phase three clinical trials assessing ceftaroline fosamil for the treatment of complicated skin and skin structure related infections and found that infections caused by *PVL* positive *S. aureus* were associated with a younger patient age for both MRSA and MSSA [37]. We hypothesize that this finding may be an affirmation of the increased virulence of *PVL* producing *S. aureus*. Host immune response is one function of the body profoundly affected by aging due to "immunosenescence" [38]. Younger adults can be assumed to be more immunocompetent compared to an older population. It would therefore take a more virulent strain of *S. aureus* to cause disease in this population. Tong *et al.* found that patients with *PVL* negative strains tended to be older and had comorbidities such as diabetes or peripheral vascular disease [37]. This group of patients is likely to have reduced host immunity and would be prone to infection even by a less virulent strain of *S. aureus*. The finding by Tong *et al.* that *PVL* is not the primary determinant in SSIs may therefore be confounded by the occurrence of *PVL* positive *S. aureus* infections in a younger population who can mount a robust immune response. Comparison of clinical outcomes in patients matched for age and comorbidities would help clarify the role of *PVL* in determining clinical outcomes.

A major limitation of this study is the relatively few numbers of *S. aureus* isolates studied especially from clinical specimens. The results of associations tested are therefore exploratory and would best be re-tested in a study powered to test these associations. However, some

of the findings are consistent with what larger studies have found.

5. Conclusion

A study specifically powered to show a difference in PVL prevalence between carriage and infective strains of *S. aureus* is required. Further studies are also required to determine the significance of the high prevalence of PVL in *S. aureus* isolates carried by healthcare workers at AKUHN and whether they contribute to hospital acquired *S. aureus* infections. The isolation of a few PVL positive MRSA isolates from clinical specimens' raises concern given their increased virulence and multidrug resistant nature as epitomized by the USA 300 strain. Continuous surveillance and appropriate infection control measures are necessary to ensure that such isolates don't become endemic in the hospital. More studies are required to define the population structure and dynamics of *S. aureus* carriage and infective strains from the region.

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