Characterization of Panton–Valentine leukocidin-positive Staphylococcus aureus from skin and soft tissue infections and wounds in Nigeria: a cross-sectional study [version 1; peer review: 2 approved]

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Abstract

Background: Staphylococcus aureus is a significant pathogen implicated in numerous nosocomial and community-acquired infections. The Panton-Valentine leukocidin (PVL) can be associated with severe necrotizing diseases such as pneumonia, skin and soft tissue infection (SSTI).

Methods: In total, 96 S. aureus isolates were obtained from patients presenting with wounds (n=48) and soft tissue infections (SSTIs, n=48). These were characterized based on their antimicrobial susceptibility profile, the possession of virulence genes (e.g. capsular type, PVL), accessory gene regulator (agr) type, and the staphylococcal protein A (spa) type. The production of the PVL protein was assessed by western blotting.

Results: All isolates were susceptible to methicillin. The resistance was highest to penicillin (97.9%), followed by trimethoprim/sulfamethoxazole (85.4%) and tetracycline (10.4%). The PVL gene was found in 83.3% of isolates from SSTIs and in 79.2% of isolates from wound. Of these, 53 (68%) produced PVL as assessed by western blotting. The most prevalent spa type was the t084 (78.1%, n=75) and, majority of the isolates carried agr2 (82.3%, n=79).

Conclusions: Prevalence of antibiotic resistant PVL-positive methicillin susceptible S. aureus strains has severe implications on PVL mediated infections.

Keywords
Staphylococcus aureus, PVL
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Author roles: Ayepola OO: Conceptualization, Writing - Original Draft Preparation, Writing - Review & Editing; Olasupo NA: Supervision; Egwari LO: Resources, Supervision; Schaumburg F: Resources, Writing - Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This study was supported by the European Molecular Biology Organization (EMBO) [ASTF 18-2011]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Ayepola OO, Olasupo NA, Egwari LO and Schaumburg F. Characterization of Panton-Valentine leukocidin-positive Staphylococcus aureus from skin and soft tissue infections and wounds in Nigeria: a cross-sectional study [version 1; peer review: 2 approved] F1000Research 2018, 7:1155 https://doi.org/10.12688/f1000research.15484.1

First published: 30 Jul 2018, 7:1155 https://doi.org/10.12688/f1000research.15484.1
Introduction

*Staphylococcus aureus* is an important human pathogen that causes significant hospital and community acquired infections. *S. aureus* producing Panton-Valentine leukocidin (PVL) is linked to a broad array of necrotizing diseases such as pneumonia and skin and soft tissue infections (SSTIs). PVL is more frequently associated with community isolates. PVL is a pore-forming toxin that can kill myeloid cells by forming channels in the plasma membrane, leading to loss of osmotic balance that ultimately lyses the cell. Earlier reports have shown PVL to be one of the most important virulence determinants in *S. aureus* from sub Saharan Africa. This study was conducted to investigate the presence of virulence genes including lukS-PV/lukF-PV, the production of the PVL protein, and the antibiotic resistance in methicillin-susceptible *S. aureus* strains isolated from wounds and SSTIs between 2010 and 2011.

Methods

Ethical statement

Ethical approval for this study was obtained from the Ethics Committee of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria (CUNG-2010-035). All participants signed a written informed consent before the commencement of the study.

Characterization of isolates

In this study we made use of an already existing database which has been published. The study was conducted in four health facilities in Ogun and Lagos States of Nigeria between June 2010 and May 2011. Samples were collected from patients presenting with SSTIs and wound infections. The isolation and identification of the isolates were done by culture and genotyping. A total of 96 *S. aureus* isolates were obtained from wounds (n=48) and SSTIs (n=48). The Vitek automated systems (bioMérieux, Marcy L’Étoile, France) was employed to determine the antibiotic susceptibility profile. The PVL gene (lukS-PV/lukF-PV), capsular polysaccharides (cap 5, cap 8), exfoliative toxins (eta, etb), the toxic shock syndrome toxin (tst) and the agr type were detected by PCR. All amplifications was done in a thermocycler (Bio-Rad, Munich, Germany). The cycling conditions and primers used was earlier published. The amplification of the lukS-PV/lukF-PV gene was carried out using primer sequences: luk-PV-1(5’-ATCATTAGTAAATGTCTGGACATGATCC A-3’) and luk-PV-2 (5’ GCATCAASTGTATGGATAACAA AAGC- 3’). The negative control was *S. aureus* ATCC 49230 (MSSA) and the positive control was sta 635/636 (a PVL-positive CA-MRSA strain). Primers specific for the variable segment of the cap locus. Cap5-f: (5’-GAAAGTGAACGATTAGT AGAA-3’) Cap5-r: (5’-GTACGAAAGCTTTGATAGTT-3’) Cap 8-f: (5’-GGGGATTTTGTAGTTT-3’) Cap 8-r: (5’-CGC CTCGCTATGAACTAT-3’) was used for the capsular typing. Sequences specific for exfoliative toxins; eta, etb and the toxic shock syndrome toxin; tst were detected by multiplex PCR. The agr types of the *S. aureus* strains were determined by the multiplex PCR strategy. Extracellular production of PVL by lukS-PV/lukF-PV –positive strains was evaluated by a Western blot using in-house antibodies raised in rabbits (anti-lukF-PV: 334 µg/ml, anti-lukS-PV: 900 µg/ml). The nitrocellulose membrane (Schleicher & Schüll, Dassel, Germany) was first incubated with rabbit anti-lukS-PV/lukF-PV antibodies (in-house antibodies, 1:1000 in TBST) and later incubated with polyclonal goat alkaline-phosphatase-conjugated anti-rabbit antibodies (1:1000 in TBST, DAKO, Germany, D0487). The membranes were washed and the bands visualized using alkaline phosphatase color development substrate (BCIP/NBT, Thermo Fischer Scientific, 34042). The production of PVL was determined semi-quantitatively in four categories: no PVL production; low PVL production, high and very high PVL production. The genetic diversity of all isolates was determined by the staphylococcal protein A (spa) typing. The highly polymorphic region X of the protein A gene, which is composed of a variable number of 24-bp repeats, was amplified by PCR. spa types were determined with the Ridom StaphType software version 1.5 beta (Ridom GmbH, Würzburg, Germany). All statistical computations were performed in SPSS Version 25. Data is explored using relevant descriptive analysis alongside chi² to measure any association between antibiotic resistance, virulence genes and lukS-PV/lukF-PV. P<0.05 is deemed to be statistically significant.

Results and discussion

We analyzed the characteristics of the PVL-positive *S. aureus* isolates as well as the relationship between antibiotic resistance, virulence genes and PVL gene (Table 1). Antibiotic resistance was highest to penicillin (100% in SSTI isolates and 94% in wound isolates), followed by trimethoprim/sulfamethoxazole (84% in SSTI isolates and 83% in wound isolates) and tetracycline (8% in SSTI isolates and 10% in wound isolates (Table 1). This is consistent with an earlier study which showed similar resistance rates for penicillin (98%), trimethoprim/sulfamethoxazole (80%) and tetracycline (18%) in Nigeria. All isolates were methicillin-susceptible. The lukS-PV/lukF-PV gene was detected in 83.3% (n=40) of SSTI isolates and 79.2% (n=38) of wound isolates. Reports from other African countries have shown high rates of PVL positive MSSA ranging from 17% to 74%. For example, a study in an Algiers hospital reported a prevalence of 72% among clinical isolates. A multi-center study reported that deep-seated SSTIs associated with the PVL gene resulted in more hospitalizations of patients and this led more often to incision and drainage. A meta-analysis showed PVL to be consistently associated with SSTIs than invasive diseases. In a study
Table 1. Association between PVL gene and antibiotic resistance.

| Antimicrobial resistance | PVL Gene | OR (95%CI) | P value |
|--------------------------|----------|------------|---------|
|                          | Absent   | Present    |         |
| Penicillin               |          |            |         |
| R                        | 16 (17.0)| 78 (83.0)  | 0.04 (0.002–0.9) | 0.003 |
| S                        | 2 (100.0)| 0          |         |
| Oxacillin                |          |            |         |
| R                        | 2 (100.0)| 0          | 23.8 (1.1–519.2) | 0.003 |
| S                        | 16 (17.0)| 78 (83.0)  |         |
| Gentamicin               |          |            |         |
| R                        | 4 (100.0)| 0          | 48.7 (2.5–955.2) | <0.001 |
| S                        | 14 (15.2)| 78 (84.8)  |         |
| Levofloxacin             |          |            |         |
| R                        | 4 (100.0)| 0          | 48.7 (2.5–955.2) | <0.001 |
| S                        | 14 (15.2)| 78 (84.8)  |         |
| Tetracycline             |          |            |         |
| R                        | 5 (50.0) | 5 (50.0)   | 5.6 (1.4–22.2)   | 0.007 |
| S                        | 13 (15.1)| 73 (84.9)  |         |
| Trimethoprim/sulfamethoxazole |  |            |         |
| R                        | 12 (14.6)| 70 (85.4)  | 0.23 (0.1–0.8)  | 0.012 |
| S                        | 6 (42.9) | 8 (57.1)   |         |
| cap 8                    |          |            |         |
| Absent                   | 5 (100.0)| 0          | 63.96 (3.3–1226) | <0.001 |
| Present                  | 13 (14.3)| 78 (85.7)  |         |
| cap 5                    |          |            |         |
| Absent                   | 13 (14.3)| 78 (85.7)  | 0.02 (0.001–0.3) | <0.001 |
| Present                  | 5 (100.0)| 0          |         |
| spa type                 |          |            |         |
| t064                     | 1 (100.0)| 0          | <0.000  | <0.001 |
| t084                     | 11 (14.7)| 64 (85.3)  |         |
| t159                     | 1 (100.0)| 0          |         |
| t194                     | 1 (100.0)| 0          |         |
| t2304                    | 0        | 6 (100.0)  |         |
| t8435                    | 0        | 4 (100.0)  |         |
| t8441                    | 3 (100.0)| 0          |         |
| agr                      |          |            |         |
| agr1                     | 5 (100.0)| 0          | NA      | NA     |
| agr2                     | 12 (15.2)| 67 (84.8)  |         |
| agr4                     | 1 (8.3)  | 11 (91.7)  |         |

Note: R=resistant, S=susceptible

carried out in Gabon, PVL-positive isolates were found to occur more in SSTIs, and PVL was also associated with resistance to trimethoprim/sulfamethoxazole.6

The presence of the PVL gene does not necessarily guarantee that the protein will be expressed and, if it is, toxin levels could vary widely from strain to strain. The production of PVL (in contrast to the sole presence of lukS-PV/lukF-PV) was observed in 75% of lukS-PV/lukF-PV SSTI isolates and 60.5% of lukS-PV/lukF-PV wound isolates. In vitro variation in the production of PVL by different strains of S. aureus has been reported and this suggests important differences in transcriptional and/or translational control of gene expression.11. In this study, the level of PVL produced by lukS-PV/lukF-PV positive S. aureus isolates varied from strain to strain (Figure 1). It was observed in that none of the PVL-positive strains harboured other toxin genes such as eta, etb and tst. Seven different spa types were identified (Table 1). The most prevalent spa type was t084 (78.1%, n=75). An earlier study revealed a significant association of the spa-CC 084 PVL-positive isolates with PVL-positive isolates.6 Typing of the agr locus, which controls the expression of many S. aureus virulence factors, showed that most isolates (82.3%, n=79) possessed the agr2, while none carried agr3. Other studies have linked isolates carrying an agr4 allele to exfoliatin-related diseases and usually carry eta and/or etb.6,19. These were absent in this study.

In conclusion, this study showed that many S. aureus isolates in Nigeria carry the PVL genes but few produced PVL in vitro. Antibiotic resistance combined with the presence of the PVL genes,
has serious implications in the treatment of \textit{S. aureus} infections. This study is limited by the few study locations. A larger study population is needed to provide a better understanding of the clones of \textit{S. aureus} in Nigeria. The results is however significant for regional surveillance.

### Data availability

Dataset 1: Results of Vitek assay, PCR results for virulence genes, \textit{agr} typing and \textit{spa} typing. 10.5256/f1000research.15484.d21182

Dataset 2: Results of PCR experiments. Gel photo for amplification of \textit{lukS-pv} and \textit{lukF-pv} gene. 10.5256/f1000research.15484.d21182

Dataset 3: Results of PCR experiments. Gel photo for amplification of \textit{agr} group. 10.5256/f1000research.15484.d21182

The results were previously presented at the 4th International Conference on Prevention & Infection Control (ICPIC 2017) Geneva, Switzerland. 20–23 June 2017. Antimicrobial Resistance and Infection Control 2017, 6(Suppl 3):52. DOI 10.1186/s13756-017-0201-4. Poster 261.

### Competing interests

No competing interests were disclosed.

### Grant information

This study was supported by the European Molecular Biology Organization (EMBO) [ASTF 18–2011].

\textit{The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.}

### Acknowledgments

The authors thank Mr Bode Onile-Ere for assistance with the statistical analysis.

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Overview
The authors characterised PVL phenotypically and also investigate the presence of gene coding for PVL production, antibiotic susceptibility and agr production. The study is interesting as it goes further from detection of PVL gene to semi quantify the product.

Introduction
Line 3, remove first ‘and’ or recast in “such as pneumonia and skin and soft tissue infections”
Spa typing is not part of the stated objectives.

Method
Characterization of isolates
“In this study we made use of an already existing database which has been published.” It should be clearly stated the data used from the referenced study above. The Method section should be rewritten so that all previously described method in Ayepola et al (2015) should not be rewritten in the present article but only referred to.

Results and discussion
It will be interesting to also expatiate on the proportion of isolates that are PVL positive molecularly and phenotypically i.e. the isolates with the genes and also producing PVL and those with the genes without PVL.
Spa typing reported in this study should be discussed too in relation to its epidemiological significance.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 13 September 2018

https://doi.org/10.5256/f1000research.16878.r37150

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Solayide Abosede Adesida
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Title
The PVL production did not appear to correlate with the presence of the gene. Therefore, the title should be modified to reflect the variability concerning the production of pvl, its detectable level, presence of the pvl genes and resistance rates.

Abstract
The authors investigated 96 S. aureus isolates were obtained from patients presenting with wounds and soft tissue infections in four health facilities in two States in Nigeria. Resistance to penicillin, trimethoprim/sulfamethoxazole was more than 80% and all isolates were susceptible to methicillin. The PVL gene was found in 83.3% and 79.2% of isolates from SSTIs and wound respectively. 53 (68%) produced PVL by western blotting. The most prevalent spa type was the t084 (78.1%, n=75) and, majority of the isolates carried agr2 (82.3%, n=79). The objective(s) of the study was not stated.

Introduction
Change “cel” to “cell”
Methods
1. There should be comma after the statement “In this study”
2. Since the isolates have been described as part of a larger study, in my opinion, extensive presentation of the previous methods used for characterising the isolates is not required. The current emphasis should be on the core findings which have not been presented in the existing or published database (ref.6). I suggest, you present a table summarizing the characteristics of the 96 isolates as obtained in the database.

Results and discussion
Information on pvl production and the presence of the genes is limited for the region under surveillance. In my opinion, this aspect of the work is relatively novel. Therefore, kindly, reconcile your findings on level of pvl production and the presence of the genes. You may also correlate these with the antibiotic resistance and perhaps the other results in the database. However, there is a lot more reports on S. aureus from skin and soft skin infection (wounds) with reference to pvl genes/production than you have cited. Please, see Sharma-Kuinkel et al¹, Zhang et al, 2018, www.nature.com/scientific reports, Hamilton et al. Clinical Infectious Diseases, 2007, 45 (2007). Other studies in Nigeria should also be considered to enrich the discussion. Overall, the discussion should be revised appropriately. However, I approve subject to the corrections suggested.

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
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Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

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