Molecular characterization of Panton-Valentine leukocidin (PVL) toxin–encoding phages from South India

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Abstract

A total of 19 methicillin-resistant Staphylococcus aureus (MRSA) isolates were investigated for Panton-Valentine leukocidin (PVL) toxin, PVL gene sequence variation and PVL-encoding phages. Whole genome sequencing was performed for all isolates. Analysis of MRSA isolates (n = 19) confirmed that most MRSA (n = 11) were positive for the PVL gene and were multidrug resistant. ST772-MRSA-V was the predominant PVL-positive MRSA clone, although all of them were found to carry the PhiND772PVL phage in the genome. This study provides insights into the evolution of a new lineage of PVL-MRSA and highlights the potential risk of the emergence of multidrug-resistant community-acquired MRSA with high virulence.

Keywords: PhiND772PVL, MRSA, PVL, ST772

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Introduction

Panton-Valentine leukocidin (PVL) toxin is a specific virulent entity, often associated with recurrent Staphylococcus aureus skin and soft tissue infection (SSTIs) and necrotizing pneumonia. Mortality due to PVL-positive necrotizing pneumonia is reported to be high (40–60%) [1]. This is due to pro-inflammatory and cytotoxic effects on neutrophils, monocytes and macrophages. Incubation of the cells with low doses of PVL (0.04–0.4 mg/mL; 1–10 nM) results in inflammasome activation, induces a huge interleukin 1β release within minutes and results in cell death (apoptosis) [2]. Despite clear epidemiologic data, the function of PVL in causing pathogenesis is controversial. Some animal models and clinical studies in bone and joint infections and necrotizing pneumonia have recognized PVL as an indicator of disease severity independent of methicillin resistance [3]. However, several other clinical trials have shown that severe SSTIs caused by PVL-producing and non producing strains do not have a difference in outcomes [4].

PVL production is encoded by two cotranscribed genes, lukS-PV and lukF-PV. The PVL encoding genes (lukS-PV and lukF-PV) are bacteriophage encoded (ΦPVL, Φ108PVL, Φ7247PVL, ΦSa2958, ΦSa2MW, ΦSLT, ΦSa2USA, ΦTCH60) [5]. PVL has been epidemiologically linked with community-acquired (CA) methicillin-resistant S. aureus (MRSA), but some CA-MRSA strains do not carry PVL genes [6]. PVL-positive hospital-acquired (HA) MRSA strains have also been reported [7].

In India, CA-MRSA clones are genetically diverse, and three-fourth are PVL positive [8]. Sequence type (ST) 772 and ST22 were the major clones reported from India [9,10]. ST772-MRSA-V, called the Bengal Bay clone, is a multidrug-resistant (MDR), PVL-positive CA-MRSA clone initially isolated and reported from India [8]. Four studies from India have reported 16% to 64% of PVL gene prevalence in S. aureus [9–12]. Despite the high incidence of PVL-positive S. aureus, clonal lineages as well the typing of PVL-encoding phages in S. aureus have not been adequately reported from India.

This study was planned to investigate the following: to analyse the distribution of PVL genes in CA- and HA-MRSA; to compare antimicrobial resistance pattern of PVL-positive and -negative MRSA isolates; to analyse mutations in PVL genes; to type PVL encoding phages; and to assess the epidemiology and molecular characteristics of PVL-positive and -negative S. aureus isolated from bloodstream infections.

Methods

A total of 19 nonduplicate MRSA isolates collected during 2015–2016 from patients with sepsis were included in this study. Antibiotic susceptibility testing was performed by the disk diffusion method for the following antibiotics: cefoxitin (30 μg), gentamicin (10 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), netilmicin (30 μg), rifampicin (5 μg), erythromycin (15 μg), clindamycin (2 μg), tetracycline (30 μg) and linezolid...
| Isolate ID | CA/HA | Source of MRSA sepsis | Antimicrobial resistance profile | Accession no | PVL gene | lukS-PVL Nonsynonymous mutation | Synonymous mutation | lukF-PVL Nonsynonymous mutation | Synonymous mutation | PVL encoding phage | SCC mec type | spa type | ST | CC |
|-----------|-------|----------------------|---------------------------------|--------------|----------|-------------------------------|------------------|-------------------------------|------------------|----------------|-------------|---------|-----|-----|
| VB9939    | CA    | SSTI                  | Gen, SXT, ery, cip              | MLQK0000000000 | 470 (T) | T | A | G | C | G | A | PhiND722PVL | V | ±57 | ST722 | CC1 |
| VB16578   | CA    | Epideral MRSA abscess | Gen, SXT, ery, cip              | MLQK0000000019 | 327 (G) | G | A | G | C | G | A | PhiND722PVL | V | ±57 | ST722 | CC1 |
| VBA46389  | CA    | SSTI                  | Gen, SXT, ery, cip              | MLQG0000000000 | 663 (T) | T | A | G | T | A | A | PhiND722PVL | V | ≤58 | ST772 | CC1 |
| VB9352    | CA    | Prostatic abscess     | Gen, SXT, ery, cip              | LXXY0000000000 | 140 (C) | A | A | PhiND722PVL | V | ±57 | ST772 | CC1 |
| VB23686   | HA    | Epideral MRSA abscess | Gen, SXT, ery, cip              | MAN0000000007 | 140 (A) | A | A | PhiND722PVL | V | ±57 | ST772 | CC1 |
| VB26276   | HA    | Necrotizing soft tissue infection | Gen, SXT, ery, cip              | LWMMF0000000000 | 527 (G) | A | A | PhiND722PVL | V | ±57 | ST772 | CC1 |
| VBA4283   | CA    | MRSA sepsis           | Gen, SXT, ery, cip              | MLQAO0000000000 | 456 (A) | A | A | PhiND722PVL | V | ±57 | ST772 | CC1 |
| VBA31683  | HA    | MRSA sepsis           | Gen, SXT, ery, cip              | MLQAO0000000000 | 140 (C) | A | A | PhiND722PVL | V | ±57 | ST772 | CC1 |
| VBA44094  | HA    | Necrotizing soft tissue infection | Gen, SXT, ery, cip              | MLQAO0000000000 | 140 (A) | A | A | PhiND722PVL | V | ±57 | ST772 | CC1 |
| VBA9982   | CA    | Necrotizing pneumonia | Cip                             | MLQAO0000000000 | 470 (T) | T | A | G | C | G | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VBA20017  | HA    | MRSA sepsis           | Cip                             | MLQAO0000000000 | 327 (G) | T | A | G | C | G | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VBA43011  | CA    | Infective spondylodiscitis | Ery                             | MLQAO0000000000 | 663 (T) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VBA1490   | HA    | STTs                  | Ery                             | MLQAO0000000000 | 140 (C) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VBA35836  | HA    | Epideral MRSA abscess | Ery                             | MLQAO0000000000 | 140 (A) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VBA35838  | HA    | Infective endocarditis | SSTI                           | LXXV5000000000 | 456 (A) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VBA21268  | CA    | MRSA sepsis           | Cip                             | MLQAO0000000000 | 140 (C) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VB1619    | CA    | MRSA sepsis           | Cip                             | MLQAO0000000000 | 140 (A) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VB35985   | CA    | MRSA sepsis           | Cip                             | MLQAO0000000000 | 456 (A) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
| VBA44746  | CA    | Necrotizing fasciitis | Cip                             | MLQAO0000000000 | 140 (C) | A | A | PhiND722PVL | V | ±57 | ST2371 | CC2 |
(30 μg). Inducible clindamycin resistance was detected by the D-zone test. Minimum inhibitory concentrations of vancomycin were determined by microbroth dilution method according to Clinical and Laboratory Standards Institute guidelines [13]. SCCmec typing was performed as previously described [14].

DNA isolation from pure cultures was performed using the QiAamp DNA mini Kit (Qiagen). The whole genome shotgun sequencing was performed for all the isolates using the Ion Torrent PGM system (Life Technologies) with 400 bp chemistry. The raw data generated were assembled de novo using AssemblerSPAdes v.5.0.0.0 embedded in Torrent suite server v.5.0.4. Genome sequence was annotated using PATRIC, the bacterial bioinformatics database and analysis resource (http://www.patricbrc.org) [15], and the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). Downstream analysis was performed using the Center for Genomic Epidemiology (CGE) server (http://www.cbs.dtu.dk/services) and PATRIC. Resistance gene profiles were analysed using ResFinder 2.1 from the CGE server (https://cge.cbs.dtu.dk/services/ResFinder). ST was determined for all the isolates in the allele order of arcc, aroe, glpf, gmk, pta, tpi and yqi by comparing the sequences with the S. aureus database maintained at the MLST website (http://saureus.mlst.net/). PHASTER (PHAge Search Tool Enhanced Release) was used for annotation and identification of prophage sequences in the bacterial genome.

Results

Of the analysed genomes (n = 19), 11 MRSA isolates were found to be PVL-positive and MDR, except two isolates. Among PVL-positive isolates, five were CA-MRSA and six were HA-MRSA. In half of the PVL-positive cases, bloodstream infection occurred as a result of SSTIs or pneumonia. All PVL-positive isolates were ciprofloxacin resistant and were often susceptible to clindamycin (D-zone negative).

The genome was analysed for single nucleotide polymorphisms (SNP) in both lukS-PV and lukF-PV genes, using the published genome of CA-MRSA strain USA300 (accession no. CP000255) as the reference sequence. Although PVL gene sequence is highly conserved, each of these genes showed nucleotide variations at three different positions. Of the identified SNPs, three were nonsynonymous (two in lukS-PVL and one in lukF-PVL). This includes phenylalanine (F) to tyrosine (Y) substitution at amino acid residue 157 (nucleotide, 470) and arginine (A) to histidine (H) substitution at amino acid residue 176 (nucleotide, 527) in lukS-PVL; and alanine (A) to valine (V) substitution at amino acid residue 47 (nucleotide, 140) in lukF-PVL. Remarkably, all PVL-positive MRSA isolates were identified as H variant (nucleotide A at 527 and histidine (H) residue at 176) (Table 1). Regardless of different MRSA clonal lineages, PVL gene variation and PVL encoding phages, the integration site (attL and attR) of these prophages seemed to be similar in all isolates.

A specific association between PVL phages and ST was observed. The Bengal Bay clone (ST772-MRSA-V) was the predominant PVL-positive MRSA clone, followed by small numbers of EMRSA clone (ST22-MRSA-IV). Among the PVL-negative MRSA isolates, clonal types were heterogeneous, including USA400 clone (ST1-MRSA-IV) and Hungarian clone (ST239-MRSA-III). It is noteworthy that the majority of PVL-positive MRSA carried the ΦIND772PVL phage in the genome, which is identified with the clonal lineage of ST722-MRSA-V and which belongs to clonal complex (CC) 1 (Table 1). Interestingly, for the first time in India, we observed ΦPVL carrying MRSA isolates belong to CC22 with the ST2371/ST22. All these ΦPVL encoding PVL toxins were identified in HA-MRSA. The phage ΦIND772PVL was found to carry PVL as well the staphylolococal enterotoxin (sea) gene, while ΦPVL was only identified with the PVL gene. spa typing showed high genetic diversity, as indicated by the presence of 11 different spa types (n = 19) among PVL-positive and -negative isolates.

Discussion

PVL toxin is considered an important marker for differentiation of HA- and CA-MRSA. The present study provides an insight into the microepidemiology of PVL-positive MRSA isolates from bloodstream infection. We observed that the majority of PVL-positive HA-MRSA are associated with SSTIs, and less likely with pneumonia or sepsis. MDR PVL-positive MRSA was observed in this study. Presumptive identification based on susceptibility to ciprofloxacin and gentamicin is no longer reliable in detecting PVL-positive CA-MRSA [16,17]. However, our study showed that all PVL-positive MRSA are ciprofloxacin resistant.

PVL gene variation identified in this study was similar to the previously described nonsynonymous SNPs [7,18–20]. In our study, all the PVL-positive isolates were identified as being of the H variant and capable of causing invasive disease. Besseyre et al. [21] demonstrated that histidine to arginine amino acid substitution does not impaired leucotoxicity of PVL toxin. Despite PVL gene variation, the H or R variant has demonstrated significant leucotoxicity. However, a possible association between other nonsynonymous mutation and leucotoxicity remains unclear. This could be an important cause
for evasion of host immunoresponsae to invasive disease [22]. Acquisition of PVL genes by an HA-MRSA strain could increase morbidity and mortality.

The PVL-positive isolates investigated in this study were from three different genetic backgrounds (ST772-MRSA-V, ST22-MRSA-IV, ST2371-MRSA-IV). This finding reveals the existence of vertical transmission of PVL genes within the same clone or horizontal transmission between different clones. The present study highlights the evolution of new lineages (ST22/ST2371) of PVL-positive HA-MRSA carrying ΦPVL phage. Interestingly, all the ΦIND772PVL phages identified in this study were found to carry PVL as well as toxin on the prophage, as previously described [8].

Four studies have reported the prevalence of PVL with clonal lineage from India. This includes two from carriers and two from clinical isolates [23]. Dhawan et al. [9] reported that PVL distribution was significantly associated with ST22-MRSA-IV (66%) compared to ST772-MRSA-V (27%) in clinical isolates. D’Souza et al. [10] reported that 65% of ST22-MRSA-IV and ST772-MRSA-V were positive for PVL, and 27% of them were MDR from mixed CA-MRSA and HA-MRSA infection. In contrast, the present study showed that ST772-MRSA-V (63%) was the predominant PVL-positive clone and was MDR.

Conclusion

Variants of the PVL gene and PVL-encoding phages are lineage specific. ST772-MRSA-V (Bengal Bay clone) belongs to CC1 and serves as a major reservoir for the dissemination of phage-mediated PVL toxin. This CA-MRSA clone (ST772-MRSA-V) was found with unique features of high virulence and MDR. In addition, co-carriage of PVL and sea toxin enhances both superantigenic and cytotoxic response. This combination of toxins on the same prophage has not been reported in other strains of S. aureus.

Conflict of Interest

None declared.

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