INSTITUT PASTEUR

Microbes and Infection 10 (2008) 878–884

www.elsevier.com/locate/micinf

Original article

The genes for Panton Valentine leukocidin (PVL) are conserved in diverse lines of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*

Carolina Berglund [a,*], Gilles Prévost [b], Benoît-Joseph Laventie [b], Daniel Keller [b], Bo Söderquist [a,c]

[a] *Department of Clinical Microbiology, Örebro University Hospital, 70185 Örebro, Sweden*
[b] *Institut de Bactériologie de la Faculté de Médecine, Hôspitaux Universitaires de Strasbourg, 3 rue Koeberlé, 67000 Strasbourg, France*
[c] *Department of Infectious Diseases, Örebro University Hospital, 70185 Örebro, Sweden*

Received 8 February 2008; accepted 28 April 2008
Available online 7 May 2008

Abstract

Methicillin-resistant *Staphylococcus aureus* isolated in the community (CA-MRSA) have been reported to carry the loci for Panton Valentine leukocidin (PVL) in high frequency. CA-MRSA in Örebro County, Sweden, constitutes at least 50% of MRSA and the PVL locus is detected in as many as 66% of these CA-MRSA isolates. The aim of this study was to characterize PVL-positive methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* by molecular methods, to determine the nucleotide sequence of *lukS-PV* and *lukF-PV* in *S. aureus* isolates of different origins, and to investigate the biological consequence of variations occurring in the genes. The PVL-positive MRSA investigated were composed of six different STs (ST8, 36, 80, 152, 154, and 256). Six additional STs (ST5, 22, 25, 30, 88, and 567) were detected when investigating PVL-positive methicillin-susceptible *S. aureus* with MLST. Despite the different genetic origins of the isolates analyzed, the PVL genes were well conserved and only one mutation was non-synonymous. Evaluation of the consequence of this mutation showed that the mutated toxin and wild-type toxin had comparable biological activity on human polymorphonuclear cells.

Keywords: Panton Valentine leukocidin; *Staphylococcus aureus*; Community-acquired methicillin-resistant *Staphylococcus aureus*; Multilocus sequence typing; Flow cytometry; Polymorphonuclear cells

1. Introduction

The presence of Panton Valentine leukocidin (PVL) in *Staphylococcus aureus* is associated with primary skin infections and necrotizing pneumonia in young, predominantly immunocompetent patients [1]. PVL belongs to the pore forming toxin family and is encoded by two adjacent open reading frames, *lukS-PV* and *lukF-PV* [2]. These genes are seldom found in the general *S. aureus* population [3], a result in concordance with a Swedish study that showed a low prevalence of PVL among *S. aureus* isolates from patients with primary skin infections, pneumonia, and staphylococcal bacteremia [4]. By contrast, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has been reported to carry the loci for PVL in high frequency [5], and to be associated with the type IV staphylococcal cassette chromosome *mec* (SCC*mec*) [6]. In Örebro County in Sweden, a low-prevalence area, CA-MRSA constitutes at least 50% of MRSA [7], and it is notable that PVL genes are detected in as many as 66% of the CA-MRSA isolates originating from this area. The presence of PVL among MRSA distributed in the community may facilitate spreading and colonization by its ability to cause primary skin infections. In addition, the cytotoxic effect against human polymorphonuclear cells and macrophages [8] is proposed to explain why PVL-positive *S. aureus* is capable of causing severe soft tissue infections and pneumonia [1].

A limited number of PVL-positive clones have been described in Europe [5], although recent reports indicate that the

* Corresponding author. Tel.: +46 19 602 1520; fax: +46 19 127416.
E-mail addresses: carolina.berglund@orebroll.se, cberglun@med.juntendo.ac.jp (C. Berglund).

1286-4579/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2008.04.018

epidemiology may be changing and that MRSA carrying the PVL genes are heterogeneous [9]. Previous studies [4,7] have indicated that PVL-positive MRSA, isolated in Örebro County, comprise several genotypes as depicted by MLST and PFGE. Also, a geographic variant of the PVL toxin have been described in MRSA from the United States [9,10] but the biological activity of this altered protein have not been investigated. Thus, assessment of the frequency, distribution, variability and function of the PVL toxin in different clones of *S. aureus* may contribute to the understanding of the significance of this virulence factor for the dissemination of PVL-positive *S. aureus* in the community.

The aim of this study was to characterize PVL-positive methicillin-resistant and methicillin-susceptible *S. aureus* by molecular methods, and to investigate if variation occurring in the PVL genes affects the biological function of the toxin.

2. Materials and methods

2.1. Bacterial isolates

A selection of PVL-positive isolates was done in order to analyze as many unrelated genotypes as possible. Isolates that were obviously related were excluded, i.e. outbreak isolates or isolates sharing the same PFGE profile. Ten PVL-positive *S. aureus* with different genetic backgrounds as determined by MLST were selected from PVL-positive methicillin-susceptible *S. aureus* (MSSA) and MRSA isolated in Örebro County, Sweden. Among these, five PVL-positive MSSA isolates identified during 1990–2002 included in a previous study [4] were analyzed by MLST in the present study and isolates with different sequence types (STs) were further investigated. Four previously investigated PVL-positive MRSA identified during 2001–2005 with different STs [7] were also included, as well as a recently isolated MRSA with a singular ST [11]. Two additional isolates were included; the MRSA CA05 [12] and one MSSA 2001-16575 [13]. Detailed information about the isolates is shown in Table 1.

In Sweden, contact tracing has been mandatory according to the Communicable Diseases Act of 2000, and essential epidemiological information has been collected and made available in county databases since 1997. Community infection was defined as previously described [7].

2.2. DNA isolation

All isolates were cultured on blood agar (Columbia II agar (BD Diagnostic Systems, Sparks, MD) 4.25% w/v; horse blood, defibrinated, (SVA, Uppsala, Sweden) 6% (w/v)) and incubated overnight at 37 °C. Automated DNA isolation was performed using the MagNa Pure LC DNA Isolation Kit III and the MagNa Pure LC Instrument (Roche Diagnostics, Mannheim, Germany).

2.3. Detection of the PVL locus, exfoliative toxins A and B, and toxic shock syndrome toxin-1

Previously described primers [4], were used in order to detect the PVL genes, employing the LightCycler® FastStart DNA Master^{PLUS} SYBR Green I and modified regarding the PCR conditions. Samples were preincubated for 10 min at 95 °C and then subjected to 35 cycles of amplification run according to the following schedule: denaturation at 95 °C for 10 s, annealing at 52 °C for 5 s, and extension at 72 °C for 18 s. Melting curve analysis was conducted by continuously registering the fluorescence while slowly raising the temperature (0.1 °C/s) from 65 °C to 95 °C.

The LightCycler® System PCR and SYBR Green I dye was used to detect the genes encoding exfoliative toxins A and B employing previously published primers [14]. Samples were preincubated for 10 min at 95 °C and then subjected to 30 cycles of: denaturation at 95 °C for 10 s, annealing at 57 °C for 5 s, and extension at 72 °C for 9 s. Melting curve analysis was performed subsequent to the amplification run.

Toxic Shock Syndrome Toxin-1 (TSST-1) was detected using previously published primers [15] and the LightCycler FastStart DNA Master SYBR Green I. Samples were preincubated for 10 min at 95 °C and then subjected to 35 cycles of: denaturation at 95 °C for 10 s, annealing at 60 °C for 5 s, and extension at 72 °C for 8 s. Melting curve analysis was performed subsequent to the amplification run.

2.4. MLST

MLST was performed with primer sequences developed by Enright et al. [16] using the real time LightCycler System PCR [7]. The STs were grouped into clonal complexes (CCs) and shared a common ancestor as predicted by the based upon related sequence types (BURST) software.

2.5. Determination of SCCmec types

SCC*mec* typing was performed using real time LightCycler System PCR according to Berglund et al. [7]. Type V SCC*mec* was detected as described [17], and the type IV (IVg) was detected using primers IVg L 5′-GCA AGC TGT TAT CGG CAT TT-3′ and IVg R 5′-GAT CGT TCG TGT TTG TGT GC-3′ as described (Berglund et al., in preparation).

2.6. Spa typing

Spa typing was performed according to Harmsen et al. [18] on the LightCycler System PCR with the SYBR Green I dye and primers 1095F 5′-AGA CGA TCC TTC GGT GAG C-3′ and 1517R 5′-GCT TTT GCA ATG TCA TTT ACT G-3′ as described (Berglund et al., in preparation).

2.7. PCR amplification and sequencing of lukS-PV and lukF-PV

Oligonucleotide primers were PVL-FW 5′-GGC AGA TTG ATC CAT CGT TT-3′ and PVL-R 5′-TGT TTG GTA ATG AAC GGG TTT-3′, and yielded a product size of 2459 bp including *lukS-PV* and *lukF-PV* and flanking regions. The PCR was performed in 50 µl reactions using the GeneAmp® PCR System 9700 (Applied Biosystems, Warrington, UK) with