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PREVALENCE OF THE PANTON-VALENTIN LEUKOCIDIN GENE AMONG CLINICAL STRAINS OF *STAPHYLOCOCCUS AUREUS*

Author. Lilian Jenny Babette SCHNEIDER (III year, Medicine).

Supervisors. Assoc. prof. Agnė KIRKLIAUSKIENĖ, lect. Lina KAPLERIENĖ, Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University; Jonas KRIŠČIŪNAS, Faculty of Medicine, Vilnius University.

Background. *Staphylococcus aureus (S. aureus)* infections are common and play a serious and important role in hospital-acquired infections. Strains of *S. aureus* with the Panton-Valentin Leukocidin Toxin (PVL) acquired higher virulence. PVL synthesis coding gene consists of *lukS-PV* and *lukF-PV* genes. The PVL generates the creation of pores in the membrane of cells like macrophages, monocytes, and neutrophils. Strains of *S. aureus,* which produce PVL, cause recurrent skin and soft tissue infections, necrotizing pneumonia, and some cases of sepsis.

The aim of the study. To determine the prevalence of PVL gene of *Staphylococcus aureus* in strains isolated from hospitalized patients in Vilnius.

Materials and methods. Clinical *S. aureus* strains were collected from 2018 to 2019. All *S. aureus* strains were stored frozen at -70°C in the Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University. The frozen strains were refreshed with Brain-Heart Agar to prepare them for the next steps. To extract the *S. aureus* DNA of the refreshed strains, distilled water and a colony from the inoculated Brain-Heart Agar were combined, heated, and centrifuged. Afterward, the extracted DNA was stored at -20°C in the Microbiology department. For detection of *lukF-PV* gene, pools of *S. aureus* lysate DNA were created. If one pool was detected positive for *lukF-PV* gene, every strain of the pool was analyzed individually. *S. aureus* strains were analyzed to detect the *lukF-PV* gene using the designed multiplex real-time polymerase chain reaction (rtPCR) protocol. As the initial rtPCR control, 16S rRNA coding sequence was applied as the DNA target. The primer and probe sequences were designed using Vector NTI Advance™ program for sequence alignment and FastPCR online Java applied for primer tests. On a total volume of 15 µl, using 1 µl *S. aureus* lysate DNA, the reactions were performed. The composition of the real-time multiplex PCR mixture was as followed: 7,5 µl 2x SensiMix™ II Probe, 200 nM concentration of each primer, and 100 nM concentration of each hydrolyzed probe. A Rotor-Gene Q 5plex HRM thermal cycler was used to perform the reactions under the following conditions: initial denaturation at 95°C for 10 minutes for one cycle, the following 40 cycles of denaturation at 95°C for 20 seconds, and primer annealing and extension at 55°C

for one minute. The detection primers and hydrolysis probes of *lukF-PV* used were as followed: Pvl_F TGGTTGGGATGTTGAAGCACA; Pvl_R TTGCAGCGTTTTGTTTTCGAG; Pvl_P HEX/TGCCAGTGTTATCCAGAGGTAACT/BHQ1.

Results. A total of 615 *Staphylococcus aureus* strains were tested for the PVL gene. 15.6% (n = 96) were isolated from blood, 13.8% (n = 85) from the respiratory tract, 65.7% (n = 404) from skin and soft tissues, 3.1% (n = 19) from urine, 1.8% (n = 11) from other specimens. Out of total isolated strains, 7.5% strains (n = 46) were PVL positive and 30.4% strains (n = 14) of PVL positive *S. aureus* were methicillin-resistant *S. aureus* (MRSA). 71,7% (n = 33) of the PVL positive *S. aureus* were isolated from skin and soft tissues, 10.9% (n = 5) from the respiratory tract, 13.0% (n = 6) from blood, 2.2% (n = 1) from urine, and 2.2% (n = 1) from other specimens.

Conclusion. 7.5% of the tested *Staphylococcus aureus* strains were PVL positive. PVL positive *S. aureus* was mostly found in skin and soft tissue specimens. 30.4% of PVL positive *S. aureus* were MRSA strains.

Keywords. Panton-Valentin Leukocidin; *Staphylococcus aureus*.