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„Szczepy grupy Staphylococcus intermedius (SIG) izolowane z materiałów klinicznych od ludzi i od zwierząt towarzyszących – identyfikacja i potencjał chorobotwórczy”

(Staphylococcus intermedius group (SIG) strains isolated from clinical samples from humans and pets – identification and pathogenic potential)

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SUMMARY

A classification and characterization of microorganisms colonizing living organisms takes into account their hosts. It is the basis for their division into typically human and animal species. However, the ranges of species colonization are not so clear. The reason of that is the constant transformation of microorganisms.

The bacteria from the Staphylococcus intermedius group (SIG) are a particular example of such an evolution. They have been regarded as able to exist only in animal organisms. This group is represented by three coagulase-positive species: *Staphylococcus intermedius*, *Staphylococcus pseudintermedius* and *Staphylococcus delphini*, which hasn't been isolated from humans yet. However, the isolation of *S. intermedius* and *S. pseudintermedius* strains from human clinical samples is rare. The actual state of their isolation frequency from humans is difficult to estimate due to the identification problems of these species. They arise from their high phylogenetic relatedness, which significantly impedes their differentiation, and from their similarity to the *Staphylococcus aureus* species, which has clinical significance. There is no required methodology used to their identification in routine diagnostic laboratories.

The topic of this doctoral dissertation was a difficult SIG species identification diagnostics and characterization of features allowing their adaptation to the human body along with the assessment of their pathogenic potential. The studies were performed on two levels: phenotypic – based on properties detection by biochemical and biological tests as well as on genotypic methods. Molecular analysis contained searching for specific genes using primers recommended in the literature as well as new species-specific primers which were independently designed in the basis of the GenBank sequence databases. In the studies were also used comparative analyzes *in silico* of the sequences of the marked genes, indicating their differences.

Initially, tested group consisted of 61 human and animal isolates from clinical materials. At first they were identified by one of the most modern techniques used in microbiological diagnostics – the MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight mass spectrometry). This method is based on the specific species profiling of bacterial proteins from cellular extracts, called „bacterial fingerprinting". In case of the tested group this method was not effective, probably because of the similarity between the model spectra of the reference strains contained in the databases. Diagnostic studies based on biochemical tests as well as commercial

identification of API Staph tests performed in the next stage did not allow for the selection of universal biochemical markers that could be used in the routine identification of these species. These studies have shown significant differences of metabolic profiles presented by the bacteria. It affects particularly the isolates collected from clinical human materials.

Molecular identification was based on s method of Sasaki et al. with Multiplex PCR for the species-specific detection of *nuc* gene encoding termonuclease. In the tested group 59 isolates were identified as *S. pseudintermedius* and two as *S. aureus*. The reliability of the method was confirmed by the identification of reference strains. In our own research on staphylococcal β-hemolysin, highly *S. pseudintermedius* species-specific primers for the *hlb* gene were designed. Their specificity was confirmed by tests with reference strains. A two-stage procedure has been suggested as a solution to the problem of routine SIG species identification due to the common and constitutive nature of β-hemolysin production among *S. pseudintermedius* strains. It would include the searching for *nuc* gene by the method of Sasaki et al. and confirmation to *S. pseudintermedius* species by detection *hlb* gene with primers proposed by the author of this dissertation.

For further studies focused on the assessment of pathogenicity, 59 *S. pseudintermedius* isolates were qualified. In this group there were 16 isolates from clinical human materials. Because of the majority of articles concerning human strains of this species describing single cases, results obtained in this dissertation have a particularly significant research value.

In the next stage, because of the possibility of interspecies transmission, the ability of the tested bacteria to colonize the host organism was evaluated. It has been shown that their ability to colonize human skin niche is determined by their rich enzymatic base (esterase, gelatinase, caseinase, lipase or CF factor) and the presence of adhesion proteins: EbpS (elastin binding protein S) and SpsE (fibrinogen/fibronectin binding protein). In the analysis of lipase gene lip and genes *ebpS* and *spsE* encoding corresponding proteins, own design primers were used. Most isolates showed the ability to form a biofilm structures, which were constructed of active cells. The competitiveness in occupying the niche and the expansiveness degree of the tested isolates were determined by their ability to produce BLIS substances and the range of inhibitory activity of these compounds against bacteria making human skin microbiota. *S. pseudintermedius* isolates showed the widespread of BLIS production capacity. The wider spectrum

of activity was demonstrated by animal isolates, but in those of human origin the activity was stronger manifested.

The toxicity profile of the tested isolates was determined by the presence of cytolysins and immunomodulatory toxins at the phenotypic level or by searching for selected genes. Their genetic equipment demonstrated in these studies mainly involved the β- and γ-haemolysin genes as well as the exfoliative toxin gene *siet*. Some isolates also had haemolysin genes of *S. aureus* that were probably obtained from this species as a result of horizontal gene transfer (HGT). The presence of Luk-I and Panton-Valentine leukotoxins genes was reported in singular isolates, but the most important fact was a detection of *pvl* gene encoding the last cytolysin. It was the first case of it in *S. pseudintermedius* species and also it is the result of HGT. No TSST-1 toxin or enterotoxin A genes were found in the tested isolates. In individual cases, both human and animal isolates were found to contain the *sec* gene encoding enterotoxin C, which is mainly detected in ruminants, but also is a factor of staphylococcus poisoning in humans.

The complex characterization of the tested isolates was supplemented by their susceptibility testing. Among the resistance mechanisms detected in the strains, the production of penicillinases and the MLS_B mechanism were particularly well represented. A mechanism of meticillin resistance, much more dangerous in a therapeutic meaning, was noted in four isolates. Determination of its molecular basis required finding the right primers for the *mecA* gene. SCC*mec* cassettes were also searched. Only four isolates were equipped with them, but they did not contain this gene. These results showed that this issue in *S. pseudintermedius* species requires further research.

The sensitivity to the basic antistaphylococcal drugs preserved in the majority of isolates creates a chance for the effectiveness of antibacterial therapy in the case of infections caused by *S. pseudintermedius* in humans. The fact of wide drug resistance occurring among animal isolates should be considered as an important element of their pathogenic potential.

The *S. pseudintermedius* species is an example of a taxon mainly associated with the animal environment, but the presented studies have shown it is increasingly isolated from clinical materials from humans. These bacteria are sufficiently equipped with factors determining colonization and human pathogenicity. Further changes of this species may contribute to perceive it as really dangerous pathogen for humans. The features of the *S. pseudintermedius* species presented in this doctoral dissertation are

the evidence of its evolutionary transformation, showing its growing pathogenic potential towards humans.