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Summary

New human infectious diseases transmitted from animals have become more noticeable in recent years. Thus, the microorganisms that cause them are monitored in more detail. Among the "animal" bacteria with various pathogenic potentials for humans, there are representatives of the *Streptococcus* and *Staphylococcus* genera, the characteristics of which were analyzed in the presented doctoral dissertation. Species selected for research are indicated as the cause of human diseases to varying degrees and frequency.

The research aim was to evaluate the selected species' ability to adapt to the human host. The study was also aimed at assessing the degree of threat from emerging pathogens. It was assumed that the adaptation process of an animal pathogen to a human is influenced by features related to colonizing abilities and the ability to invade. The determinants (markers) that could influence this adaptation were sought. The aim was also to investigate what therapeutic options a physician may have in case of human infection with these strains.

All 93 strains studied in this work have been genetically identified. Previous identification of *Streptococcus* strains from human clinical specimens by MALDI TOF MS was genetically confirmed using the 16S rRNA primer sdys for *S. dysgalactiae* and the tanB and sgp primers for *S. gallolyticus* subspecies. Species rarely isolated from humans: *S. uberis*, *S. parauberis* and *S. dysgalactiae* subsp. *dysgalactiae* was grown and selected by analyzing 660 bovine mastitis milk samples and identified phenotypically and genotypically using the pauA primers for *S. uberis* and spa2152, spa2870 for *S. parauberis* identification. For strains new to this collection, *S. dysgalactiae* subsp. *dysgalactiae* obtained by sequencing the 16S rRNA gene, the sequences were placed in the GenBank database. These strains were placed in the phylogenetic tree based on percent identity with other sequences identified at the subspecies level. In the case of the genus *Staphylococcus*, 14 strains of *S. pseudintermedius* isolated from both animal and human clinical materials were analyzed. They came from department collection and had been previously identified genetically. To assess the phylogenetic relationship, the housekeeping genes of these strains were sequenced, and MLST analysis was performed. Sequences were also placed in the GenBank database.

In the study, growing methods and metabolic and biological properties were used. Genes were searched by PCR based on sequences derived from *S. pyogenes* and *S. dysgalactiae* in streptococci and *S. aureus* and *S. pseudintermedius* in staphylococci. In selected strains of the genus *Streptococcus*, the expression of some genes of invasins, streptokinase, complement peptidase C5a, streptococcal complement inhibitor and phospholipase A2 was studied.

Among the assessed metabolic features of streptococci, caseinase and broad saccharolytic abilities, including sorbitol degradation, were characteristic of strains isolated from animals. Among this group of strains, the gene encoding hyaluronan lyase (*hylB*) was rare,

characteristic of strains isolated from humans. ECM adhesion genes responsible for colonizing the studied streptococci (*hylB*, *fbp*, *cbp* and *prtF1*) were more numerous in *S. dysgalactiae* strains than in other streptococcal species. In *Staphylococcus pseudintermedius* strains, genes responsible for ECM binding and colonization were detected in a similar number in "human" and "animal" strains. Still, only strains isolated from animals had *spsP* and *spsQ* genes.

In the assessment of the invasiveness of the strains, special attention was paid to streptokinase. The *ska* gene encoding it was present in all *S. dysgalactiae* and numerous strains of other species, but it was not expressed in all strains examined. The activation level of human and bovine plasminogen was also differentiated, showing that this feature can be considered in assessing potential adaptation to humans as a new host. A feature that significantly differentiated "human" and "animal" strains, but not species, was the ability to grow in the presence of bovine and human serum. Only in the genomes of strains of *S. dysgalactiae* subsp. *equisimilis*, isolated from humans, the streptococcal complement inhibitor gene *sicG* was found, but the DNase gene *sdn* was found only in strains of the species isolated from animals.

Among the tested streptococci isolated from animals, nine strains were selected whose pathogenicity profile was similar to human strains, and among those isolated from humans, twelve strains were selected that retained characteristics similar to "animal" strains. Further study on these strains, including whole genome sequencing and M protein typing, may yield significant results for their adaptation to the human body.

The strains of *S. gallolyticus* differed in their characteristics from the others in many respects. With metabolic features similar to strains isolated from animals (the ability to decompose casein and a broad profile of sugar decomposition), they had *isp1* and *dppA* genes, common to strains of *S. dysgalactiae* subsp. *equisimilis*.

Genes of leukotoxins and exfoliatins were common among *S. pseudintermedius* strains of both studied groups. Only strains isolated from humans had cell surface-bound factor CF.

Phenotypic (disc antibiogram, E-tests) and genetic drug susceptibility assessment of the tested *Streptococcus* species indicated their sensitivity to many antibiotics. However, frequent resistance to macrolides, lincosamides and tetracyclines was observed. Many *S. pseudintermedius* strains were multidrug resistant.

A large variety of pathogenicity and drug susceptibility characteristics was observed in the strains of each studied species. This confirms the thesis of their very wide pangenome. The accumulation of traits conducive to breaking the barrier of a new host in single strains, followed by their clonal selection, may lead to the emergence of a new human pathogen. After analyzing the results obtained for the tested strains of streptococci and staphylococci, it can be assumed that the colonizing ability expressed in the production of ECM binding proteins is not a feature of key importance. On the other hand, the most important for overcoming this barrier in streptococci are the features that allow opposing the actions of the immune system, including the C5a complement peptidase encoded by the *scp* gene and the streptococcal complement inhibitor by the *sicG* gene. The expression of the *dppA* gene, which regulates the action of the streptococcal toxin, and the *isp1* gene, which regulates many streptococcal toxins, including the

M protein, may also be important. However, none of these genes can yet be indicated as a marker of adaptation to the human body. In the case of staphylococci, the species *S. pseudintermedius* seems to be well adapted to the human body, and further research should focus on the epidemiology of its infections, which would allow us to determine the length of the transmission chain of pathogenic strains between people. The presented doctoral thesis indicates the directions of further research, the result of which may contribute to explaining the process of adaptation of animal pathogens to the human body.