Wydział Farmaceutyczny
Katedra Biologii i Biotechnologii Farmaceutycznej
Zakład Mikrobiologii Farmaceutycznej i Diagnostyki Mikrobiologicznej

mgr farm. Marcin Ciszewski

Czynniki chorobotwórczości *Streptococcus dysgalactiae* – paciorkowców ropotwórczych izolowanych od ludzi i zwierząt.

Rozprawa na stopień naukowy doktora nauk farmaceutycznych.

Promotor: Prof. dr hab. n. farm. Eligia M. Szewczyk

Łódź 2016

STRESZCZENIE W JĘZYKU ANGIELSKIM

Streptococci constitute a large and very diversified group of bacteria, comprising both human and animal pathogens. Among this Gram-positive genus, an A group streptococcus - *Streptococcus pyogenes*, a human-exclusive pathogen, has been most thoroughly studied. Many other streptococci species are isolated solely from animals, whereas some species are subjected to various evolutionary changes enabling their animal-to-human transfer, and as a result they become etiological factor of zoonotic infections. Streptococcal zoonoses comprise usually mild infections. However, systemic and fulminant ones, which might lead to septic shock and death, have been also described for some species. *Streptococcus dysgalactiae*, analyzed in this dissertation, comprises two subspecies: *S. dysgalactiae* subsp. *equisimilis* (SDSE) and *S. dysgalactiae* subsp. *dysgalactiae* (SDSD). Until recently, both subspecies have been considered as exclusive animal pathogens causing infections in livestock as well as in companion animals. Currently, human infections, including severe ones, are being reported with increasing frequency and their clinical picture is often similar to the ones caused by *Streptococcus pyogenes*.

The main aim of this study was to analyze virulence potential and virulence factors of *Streptococcus dysgalactiae* strains isolated both from human and animal clinical cases, and afterwards, to assess certain virulence features that might enable *S. dysgalactiae* strains to transfer from animal to human.

In the presented study various classic and instrumental research techniques have been used, comprising both phenotypic and genotypic analyses. Identification of bacterial strains to the subspecies level was performed by means of MALDI-TOF technique based on protein profiles analysis, RISA – genetic analysis based on subspecies-specific DNA sequences, as well as 16S rDNA sequencing. A comprehensive virulence factors analysis was performed, including classical bacteriological methods, serological tests, searching for 26 virulence genes along with their expression analysis, as well as an evaluation of biofilm formation ability and BLIS substances production which inhibit growth of human skin microbiota. Those tests were complemented with innovative *in silico* analyzes of SDSE and *S. pyogenes* genomes deposited in Genbank. The data obtained in *in silico* analyses, confronted with results obtained in genetic studies, allowed to evaluate a

possible mechanism of virulence gene transfer between *S. dysgalactiae* strains, and from *S. pyogenes* to *S. dysgalactiae*.

Subspecies identification constituted the first challenge in the presented research. The results of molecular strains identification have been compared with phenotypic subspecies division criteria described by Vandamme *et al.* in 1996 and Vieira *et al.* in 1998. The criteria proposed by both research teams, as well as results of performed molecular identification are contradictory. According to contemporary phylogenetic criteria, which base on genetic relatedness, all analyzed *S. dysgalactiae* strains were classified as SDSE. The obtained results suggest that formerly established subspecies division criteria should be redefined or rejected completely, admitting that their characteristics fit in an open pangenome of *S. dysgalactiae* species. A concept which suggest replacing *S. dysgalactiae* division into subspecies with division into patotypes, which are characterized by the ability to colonize different hosts and their potential to cause infections of certain clinical picture, is also worth considering.

The detailed results of phenotypic and genotypic virulence factors evaluation are included in the Results section, as well as in 15 tables of the Annex section of this dissertation. A number of virulence factors, taking part in adhesion to host cells, colonization, infection spreading and evasion of the host immune system, were identified in SDSE human isolates. The presence of aforementioned virulence factors and the fact that those strains were isolated from clinical cases where active medical intervention, in hospital or ambulatory, was required, proves that they were well adapted to human organism colonization. SDSE animal isolates were significantly less equipped with virulence factors. None of the analyzed animal isolates possessed an ability to produce streptokinase or genes coding for M protein, streptolysin O and fibronectin-binding protein. Abovementioned virulence factors were present only in human isolates, what might suggests that the presence of these factors conditions the SDSE strain ability to colonize human organism and to cause symptomatic infection. All analyzed strains had an ability to form bacterial biofilm, an organized community present both on skin and mucosal membranes, as well as on artificial surfaces, including medical equipment. This feature indicates a high colonization potential of this species. However, only human isolates had an ability to produce BLIS (bacteriocin-like inhibitory substances) which inhibit growth of *Corynebacterium* spp. on human skin and as a result allows to overcome the colonization resistance of human skin microbiota.

Antimicrobial susceptibility tests of SDSE strains, conducted in this research, showed that a significant percentage of strains were resistant to tetracyclines, macrolides and lincosamides. Human SDSE isolates resistant to penicillin, vancomycin, linezolid and tigecycline were also noted. This high percentage of resistant strains, especially in species that cause relatively rare infections in humans, indicates that resistance might be acquired at the time when animal hosts were colonized, before animal to human transfer. The main cause of this situation might be an extensive, often overused, empirical antibacterial treatment in veterinary medicine.

Substantial differences in virulence genes presence and expression of virulence factors in human and animal isolates, hinder an unambiguous current evaluation of *S. dysgalactiae* position in the outline described by Wolfe *et al.* comprising evolutionary changes of animal pathogen which result in becoming solely human pathogen. Symptomatic human infections are most likely not caused directly after strain transfer from animal to human. Animal strains are successively transferred to human organism, enriching its microbiota. That is an intermediate stage of temporary or permanent colonization – strain colonizes human skin or mucosal membranes, without causing any symptoms of disease. At that time, it might receive virulence genes from other commensal human streptococci or *S. pyogenes* pathogenic strains. The symptomatic infection occurs only after tissue damage, immunological impairment or other conditions favoring opportunistic infections.

In comparative genomic SDSE and *S. pyogenes* analysis, based on genome sequences deposited in databases, the virulence "pangenome" of these species has been obtained. This allowed to observe sets of genes, located in relatively close genomic positions, that potentially could be transferred together via a horizontal gene transfer. This transfer could definitely occur between SDSE strains, as well as between species, from *S. pyogenes* to SDSE. An example of this kind of gene group contains: *ska* (streptokinase), *scpA* (C5a peptidase), *smeZ* (streptococcal mitogenic exotoxin Z) and *lmb* (laminin-binding protein).

The obtained in this dissertation results attract attention to the vital problem of classification of the microorganism isolated in medical laboratories with increasing frequency. Due to the high genome plasticity and streptococcal genetic variability, the presented in this dissertation data suggest that in non-distant future *S. dysgalactiae* strains might become clinically important human pathogens.