Summary

Bacteria of the *Staphylococcus* genus are a large group of microorganisms that are well-known commensals and can cause various infections in companion animals, farm animals, and wildlife animals, as well as humans. Coagulase-positive *Staphylococcus* species (CoPS) colonize the skin, mucous membranes, particularly the upper gastrointestinal and respiratory tracts, and the genitourinary tract.

The widespread use of antibacterial substances, both in therapy, metaphylaxis, and prophylaxis in animal production, has significantly increased the risk of the spread of drug-resistant microorganisms, including bacteria of the *Staphylococcus* genus. Therefore, in recent years, the increasing drug resistance of microorganisms, including coagulase-positive *Staphylococcus* species, has become one of the greatest challenges in veterinary medicine. Currently, particular attention is being paid to methicillin-resistant *Staphylococcus* isolates, which often also exhibit multidrug resistance, i.e., resistance to at least three antibiotics from different antimicrobial classes.

The aim of the research conducted in this study was to assess the prevalence of coagulase-positive *Staphylococcus* in host animals varying in species, therapeutic range, and health status, with a view to comparative analysis and assessing the level of drug resistance of these microorganisms, as well as the virulence factors responsible for *Staphylococcus* related infections. In addition to assessing the public health threat posed by multidrug-resistant and/or methicillin-resistant bacteria possessing a broad spectrum of virulence factors, the study aimed to determine the extent to which individual, epidemiologically significant bacterial clones spread between different animal species. Due to their widespread occurrence in various hosts as commensals and, at the same time, infectious agents of numerous diseases with diverse clinical presentations, coagulase-positive *Staphylococcus* bacteria constitute an extremely interesting and dynamic group of microorganisms, which, despite numerous studies, continues to present new challenges. Therefore, one of the research goals was to attempt to explain the interaction between the host's immune response to the presence of these bacteria by analyzing the expression of genes involved in this process. In the latter case, the target research group were small ruminants, and the aim of the study was to analyze the expression profile of cytokine genes: *IL-1α, IL-1β, TNF-α, IL-6* and acute phase protein genes *SAA*, *Hp* and *Cp* in two groups of clinically healthy sheep: animals carrying CoPS, taking into account their virulence factors, and sheep in which the presence of CoPS was not detected.

The overall analysis of the drug resistance profile showed that the majority of CoPS strains were resistant to tetracycline (n=160), penicillin (n=141), clindamycin (n=128), and erythromycin (n=117). The phenotypic resistance profile generally correlated with the presence of genes, primarily *tet*M and *bla*Z.

Relatively high rates of resistance to oxacillin and cefoxitin were also observed, depending on the CoPS species, primarily in dogs (n=4 *S. aureus* and n=17 *S. psudintermedius*) and sheep (n=5 *S. aureus*). In both groups of animals, strains were isolated from various sites, primarily the oral mucosa. Furthermore, three methicillin-resistant *S. aureus* strains isolated from sheep and dogs also demonstrated resistance to vancomycin.

The study also revealed a rich profile of virulence genes. However, the majority of *S. aureus* strains isolated from all animal groups, including wildlife animals, possessed the *se*B virulence gene, encoding one of the enterotoxins (n=76), and *Luk*E-*Luk*D, encoding the *LUKE/LUKD* leukotoxin (n=63). In the case of *S. pseudintermedius*, the majority of strains derived exclusively from companion animals possessed the *se*L gene (n=69). However, it is worth noting that genes such as *luk*S-*luk*F, encoding leukocidin components, *ica*A and *ica*D, encoding biofilm components, *siet*, encoding an exfoliative toxin, and *Sec canine*, responsible for encoding one of the enterotoxins, occurred almost equally frequently (68 strains each possessed the previously mentioned virulence gene). Furthermore, MLST analysis identified new STs (sequence types) in three MRSA strains isolated from sheep (ST 9313, ST 9314, and ST 9315), two MRSA isolates from dogs (ST 9311, ST 9312), and four *S. pseudintermedius* isolates (ST 2739, ST 2740, ST 2741, ST 2742), also from dogs. Furthermore, strains isolated from farm animals and strains isolated from companion animals were analyzed using *ADSRRS*-fingerprinting to compare their genomic profiles. This method proved useful for strains isolated from sheep, indicating the spread of strains with the same genotype among host animals belonging to the same flock. In small ruminants, additional studies were conducted comparing the expression of four cytokine genes and three acute-phase protein genes in leukocytes from sheep carrying *S. aureus* and those without *S. aureus* (control): *IL-1α*, *IL-1β*, *IL-6*, *TNF-α*, *SAA*, *Hp*, and *Cp*. A statistically significant increase in both IL-6 and Hp during pregnancy was observed in leukocytes from Uhruska sheep carrying *S. aureus*. Similar trends were observed in Świniarka sheep, but the differences were not statistically confirmed.

Summarizing the results of the obtained studies, it was demonstrated that farm animals, companion animals, and wildlife animals remain an important reservoir of coagulase-positive strains, i.e., *S. pseudintermedius* and *S. aureus*. The analyses also showed that regardless of the

animal species, the most predilection site for CoPS is the oral mucosa. It is important to emphasize that in many cases, the same host can harbor several CoPS strains belonging to the same species, differing in genome, including resistance and virulence profiles, as well as sequence type. It is worth noting that wildlife animals, despite theoretically low selection pressure due to the lack of targeted therapy, also constitute a source of drug-resistant and virulent CoPS strains. In the case of companion and farm animals, the presence of MRSA, MRSP, vancomycin-resistant strains, and multidrug-resistant strains is particularly noteworthy. Given their close contact with humans, these reservoirs represent a source of strains posing a potential threat to public health. Furthermore, the rich virulence gene profile in both species, particularly manifested by the presence of genes encoding enterotoxins, exfoliative toxins, and toxic shock toxin, which possess superantigenic properties, may further promote the development of severe infections in both hosts and humans. The identification of new sequence types (STs), particularly among MRSA and MRSP strains, and the increasing dominance of STs, previously of little epidemiological significance, indicate the continuous evolution and development of new adaptive mechanisms by CoPS strains to new hosts and environments. This, coupled with the rich panel of resistance and virulence factors, makes this group of bacteria a still largely unknown threat to public health. Moreover, despite the confirmed commensal status in animals, the presence of CoPS species, even without generating clinical symptoms of infection, activates the host immune system, as evidenced by statistically significantly higher levels of IL-6 and Hp in *S. aureus* carriers, especially in pregnant animals.

Keywords: Coagulase-Positive *Staphylococcus*, Drug Resistance, Virulence, One Health, Animals