PROPERTIES OF COAGULASE-POSITIVE STAPHYLOCOCCAL CELLS THAT MAKE IT DIFFICULT TO DIAGNOSE AND TREAT MASTITIS IN COWS

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Abstract

Coagulase-positive staphylococci are a group of bacteria that, among other things, cause inflammation of the udder in cows (mastitis). The identification of the species of staphylococcus causing the inflammation is crucial for the success of the treatment process due to the individual characteristics of the strains and even the characteristics of the bacterial population in the herd. However, through evolution, staphylococci have developed a number of processes that make species identification difficult. Knowledge of the actual cause of inflammation enables the selection of appropriate therapy, but despite advanced diagnostic techniques, erroneous results still occur. In this review, we present the current knowledge of mainly resistance mechanisms and how they affect the drug resistance of microorganisms. We also highlight the difficulties in the diagnosis and treatment of bovine mastitis.

Running title: Mastitis in cows caused by coagulase-producing staphylococci

Keywords: mastitis, cows, diagnosis, *Staphylococcus aureus*, coagulase-positive

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Introduction

Bacteria of *Staphylococcus* were divided into coagulase-positive (CPS - Coagulase Positive *Staphylococci*) and coagulase-negative (CNS - Coagulase Negative *Staphylococci*). This division was due to the features of metabolism, parts of bacteria that allow the production and secretion of an enzyme called coagulase into the external environment. By infecting the cow's udder, staphylococcal bacteria initiate a reaction of the immune system. First, there is a non-specific response of the organism in the form of increasing the temperature of the udder. Then the pathogen is attacked by the appearing granular cells in the place of demand thanks to the concentration gradient of chemoattractants such as the CXCL8 chemokine or by the use of cell surface receptors by, for example, leukotriene B4 (LTB4) for the recruitment of granulocytes [1]. Excessive amount of leukocytes increases the number of somatic cells in milk - making it possible to notice infection. The infected cow becomes a reservoir of the pathogen in the herd, making it possible to transmit bacteria to the other cows.

Discussion of the problem

In the structure of their walls, staphylococci possess proteins (FnBPA - fibronectin-binding protein A) that have a role in the adhesion process, but FnBPA also has a variable affinity for fibrinogen [2]. In addition to proteins such as FnBPA, coagulase also acts to bind fibrinogen. The described activity of this enzyme comes down to the formation of a capsule around the bacteria. The capsule protects the bacterial cell against the activity of the immune system of the infected organism [3]. Regulation of coagulase production takes place in the region *agr* however, as some studies indicate, there may be a targeted inhibition of the activity of this part of the genotype in order to avoid the reaction of the immune system of the infected organism [4]. In addition, a possibility of the formation of a biofilm by staphylococci, a group of bacteria coated with polysaccharide intercellular adhesin (PIA), makes it difficult to treat infections with these microorganisms. Covering the tissue with biofilm is not only beneficial due to the possibility of living close to epithelial cells, but it has also been proven that the structure of the biofilm retains water and nutrients for bacterial cells [5]. There are reports of the degradable influence of the active form of artesunate - dihydroartemisinin, a widely used agent in the treatment of malaria, on biofilm of the *Staphylococcus aureus* [6]. However, *Staphylococcus aureus* bases its antibiotic resistance on the production of enzymes - beta-lactamases. They are coded by the gene *blaZ*. They are proteins that hydrolyze antibiotics from the group of beta-lactam antibiotics. Reported frequency of occurrence of the *blaZ* is variable depending on the region. The data for the

USA and Germany are similar, as they are at the level of 41.2% and 47.1%, respectively [7]. On the other hand, information showing this frequency in Brazil is 74.07% [8]. *Staphylococcus aureus* borderline resistant to oxacillin, an antibiotic of the beta-lactam group, is described. It is most likely related to a mutation in the gene *blaZ* or a change in the structure of PBP2 (Penicillin-binding protein) [9]. These data demonstrate the continuing variability of microbes in terms of antibiotic resistance. *Staphylococcus aureus* regularly resistant to beta-lactam antibiotics is called MRSA (Methicillin-resistant *Staphylococcus aureus*). The pathogenicity of MRSA is guaranteed by the presence of the gene in the mobile part of the bacterial genotype *mecA*. The presence of this gene in coagulase-positive staphylococci is region-specific. In South-West Asia, the values presented by the authors are much higher than in European countries. One such country is Iran where the presence of the gene *mecA* has been shown to be 35% [10]. Another example is the study from Pakistan where the gene *mecA* occurred with a frequency of 30% [11]. For comparison, in Germany and Italy its incidence is much lower (6.9% and 5.9%, respectively) [8]. A higher rate for European countries is reported in a study on the prevalence of the *mecA* gene in Greece, which was 10% there [12]. MRSA is characterized by a specific configuration of PBP2, a cell wall protein that has a low affinity for penicillin. However, also in this case there is considerable variability in resistance to the individual antibiotics of the beta-lactam panel. This is indicated by the data from the study Fergestad ME (2020) talking about the highest resistance to cefaclor and cefazolin [13]. The resistance profile of *Staphylococcus aureus* is variable, as indicated by the conducted studies [14]. Namely in one of the studies Mphahlele MP (2020) presents data on 2,862 samples from which 50 *Staphylococcus aureus* were isolated. Each of them was resistant to at least one of the antibiotics to which they had been exposed. In contrast, 62% were resistant to three or more categories of antibiotics. They were most often resistant to erythromycin (62%), ampicillin (62%) and oxacillin (46%) [15]. On the other hand, Bissong MAE (2020), analyzing the resistance of *Staphylococcus aureus*, showed 39 isolates *S. aureus* from 289 microorganisms [16]. In both of these studies, staphylococci were characterized by high resistance to erythromycin (82% - Bissong, 62% - Mphahlele), and in the case of Bissong MAE work, there was high resistance to vancomycin (80%) and tetracycline (76%). These data are worth comparing with the Egyptian report of much lower resistance of *Staphylococcus aureus* for antibiotics. Namely, resistance to erythromycin was 30.9%, to tetracycline 59.5% and to penicillin 64.3% [17]. A study by Shi C. (2021) presents the prevention and resistance profile of *Staphylococcus aureus* to antibacterial preparations. The study showed that 5

out of 15 used antibacterial agents were isolates of *Staphylococcus aureus* possessed over 50% degree of resistance. The bacteria that were the least sensitive turned out to be penicillin G (81.5%), sulfamethoxazole (73.6%), trimethoprim (70.3%), oxacycline (63%), and clindamycin (50.4%). It is worth adding that different results have been noticed in different parts of the country regarding the average rate of resistance. The highest percentage of infections was recorded in the northern part of the country, while the lowest in the southern part. On the other hand, the incidence of *Staphylococcus aureus* in research samples was 36.8% [18].

Other staphylococci, due to the significant dominance of the *Staphylococcus aureus* in the percentage of infections, were referred to as Non-Aureus Staphylococci (NAS). Coagulase-positive bacteria causing mastitis in NAS cows in cows belong to NAS are *S. agnesi*, *S. hyicus*. One of the studies presented the prevalence of *S. hyicus* less than 6.5% [19]. Diagnosing the staphylococcus species with NAS is much more difficult compared to diagnosing *S. aureus*. This is due to the multitude of variants and variability of this group of bacteria, and also due to a much more detailed understanding of *S. aureus* by researchers. Reliability of the examination of the diagnosis of coagulase-positive staphylococcus species other than *S. aureus* gives the analysis of isolates using the MALDI-TOF MS technique (Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry) consisting in pulsed laser irradiation of a properly prepared research material and then ionization by being protonated or deprotonated. The analyzed spectrum gives a high probability to determine the species and their proteins in a short time [20]. Typing a staphylococcal species on the basis of its phenotype is a much less accurate method. One of the studies reported results where 52 out of 131 (40%) isolates were correctly identified (according to the results of the MALDI-TOF MS method). An interesting observation was the fact that 48% of the tested samples were not identified when examining the phenotype [21]. This is due to the greater phenotypic lability of staphylococci [14]. Due to the metabolic properties of the coagulase positive staphylococcal bacterial cell, it is possible to judge whether the inflammation is staphylococcal because coagulase is a rare enzyme. This is important because a significant percentage of inflammation in the mammary gland is caused by coagulase-positive staphylococci. However, some studies show the presence of bacterial cells of coagulase-positive staphylococci species that are unable to produce coagulase. Such a species is e.g. *Staphylococcus hyicus* - this bacterium is called coagulase-variable. There are also reports of coagulase variability of *Staphylococcus aureus* [10]. In this study Saeed Shahmohammadi (2019) presented 200 isolates of *Staphylococcus aureus* of which 143

(71.5%) were coagulase-positive and the rest did not clot in the plasma. However, these data may not be consistent with reality due to the facts presented in the study by Almeida K. (2018). This study shows the percentage of false positive tests that diagnose the *Staphylococcus aureus*. Out of 408 samples, 32 *Staphylococcus aureus* strains were obtained after a positive coagulase test and Sa442 PCR. However, after the latex agglutination test, the number of true strains belonging to this species was reduced to 10, and 8 remained after passing the MALDI-TOF MS test. Additionally, a RealPCR study was conducted which reduced this number to 7. Most commonly, the coagulase-negative bacterium *S. chromogenes* was misidentified as *S. aureus* [22]. Gene sequencing in diagnostics allows for almost 100% determination of *Staphylococcus*. This is demonstrated by the results of the study by Wanecka A. et al. (2019) where the genetic material was sequenced the *dnaJ* and *rpoB* genes. This method allowed for the correct identification of all species [21]. Sequencing methods used to mark the genes of the microbes causing mastitis are: ribotyping [23], pulsed-field gel electrophoresis (PFGE) [24], multilocus sequence typing (MLST) [25], amplified fragment length polymorphism (AFLP) [26], random amplified polymorphic DNA (RAPD) [27].

The occurrence of infections with coagulase-positive staphylococci is not only dangerous to the health and life of cows, but also to consumers, due to the possibility of accumulating enterotoxins in milk [28]. The genes responsible for secreting enterotoxins are also detected with different frequency. The Fursov study carried out in Russia showed that the most common gene from the enterotoxinogenic panel was *seg* (70%). The group of the most frequent also includes the *sea* (53.3%) *see* (46.6%) *ise* (50%), while the *sed* (4%) was rare [29]. In another study, the presence of the *sea* (35.29%) was at a lower level. The *sec* gene was much less frequent (5.88%) and there was little change in the prevalence of the *see* gene (47.07%). Instead, the prevalence of the *sed* gene changed significantly (29.41%) [30]. In the treatment of mastitis, intramammary antibiotic therapy is often selected [31, 32]. However, the presented data on antibiotic resistance and the processes of acquiring resistance in bacterial cells necessitates the search for alternative methods of mastitis treatment. One of the studies compared the effectiveness of antibiotic therapy and non-antibiotic therapy in the treatment of clinical mastitis [33]. The medicinal product was supplied as an intramammary syringe containing 120 mg α -tocopherol acetate, 58.83 mg retinol palmitate, 2,400 FIP-U chymotrypsin, 240 FIP-U trypsin and 6 FIP-U papain. This study compared the probability of the mastitis coming back 60 days after healing. In the group treated with antibiotics alone, this rate was 53.6%, while the probability of

inflammation recurrence in the group of cows treated with the non-antibiotic preparation was 52.2%. These results indicate that treatment of non-severe cases of clinical mastitis with this preparation can be an alternative to antibiotics. Vaccination is another route of therapy to reduce the incidence. This approach is based on allowing the immune system of animals to fight pathogens effectively. Polyvalent vaccines to prevent mastitis are widely reported [34, 35]. One of the studies presented the effect of a product containing an inactivated strain of *S. aureus* (CP8) SP140, which stimulates the immune system [36]. The results of the study indicated that the immune system of the vaccinated subjects was better able to cope with infection with the strains *Staphylococcus aureus* than unvaccinated individuals. On the other hand, another study proposed the use of nisin Z, along with inactivated bacteria isolated from the farm [37]. This solution resulted in a reduction in the number of somatic cells in milk and a simultaneous increase in milk fat and protein compared to the sample devoid of nisin Z.

Conclusions

Staphylococci are a very specialized and dangerous group of bacteria which, thanks to the unique properties of their cells, make it difficult to diagnose and treat diseases caused by them.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest statement

The authors declare they have no conflict of interest.

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