Animal Science Papers and Reports vol. 35 (2017) no. 2, 123-135
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Current concepts on the impact of coagulase-negative *staphylococci* causing bovine *mastitis* as a threat to human and animal health – a review*

Grażyna Sender[1], Adrianna Pawlik[1], Agnieszka Korwin-Kossakowska[1]**

[1] Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzębiec, 05-552 Magdalenka, Poland

(Accepted January 9, 2017)

Staphylococci **are the main aetiological factor of bovine** ***mastitis*** **in many dairy herds. Traditionally, coagulase-negative** ***staphylococci*** **(CNS) were considered a normal skin microbiota. Lately the role of CNS in bovine mastitis has increased, as in some countries these pathogens had started to outnumber other mastitis aetiological factors. Given the scale of** ***mastitis*** **problems, the intensive use of antibiotics in dairy cattle, the number of animals and the consumption of milk products there is an urgent need to highlight the threat to both human and animal health originating from CNS. The knowledge of how CNS** ***mastitis*** **develops, spreads in herd and persists is limited. Bovine strains of CNS are poorly characterised; moreover, they are characterised mainly in terms of phenotypes. This review summarises knowledge on the characterisation of CNS strains, stressing the role of the bacterial genotype, in the context of the risk to human and animal health. Selected CNS virulence factors that play a role during** ***mastitis*** **in dairy cattle are listed and described in this paper. They have been selected subjectively by the authors in view of their significance for public health (toxins, antimicrobial resistance) and their importance for animal health (formation of biofilm, prevention of phagocytosis intracellular survival) and** ***mastitis*** **outcome.**

KEYWORDS: bacterial genotype / dairy cattle / ***mastitis*** **/** ***staphylococci***

Staphylococci remain the main aetiological factor of bovine *mastitis* in many dairy herds. Traditionally, coagulase-negative *staphylococci* (CNS) were considered normal skin microbiota and they were isolated *e.g.* from the skin of the udder and teat

*The publication was funded within the project No. 2011/01/N/NZ9/00567 financed by the National Science Centre
**Corresponding author: a.kossakowska@ighz.pl

canals. Until recently CNS strains associated with bovine *mastitis* were frequently regarded as minor bacteria and ignored in research. Since then the pathological role of CNS in bovine *mastitis* has increased inevitably in many countries. Simojoki *et al*. [2011] stated that in Finland the prevalence of *mastitis* caused by major pathogens [*Staphylococcus aureus* (*S. aureus*)*, Streptococcus agalactiae* (*S. agalactiae*) *and Escherichia coli* (*E. coli*)] decreased, while the incidence rate of CNS-caused *mastitis* is escalating and starts to outnumber the figures for all other bacteria. In Poland CNS are responsible for about 14.6% of mastitis cases [Sender *et al*. 2003, Malinowski *et al.* 2006, Sender *et al.* 2006] and are found in 44.8% milk samples [Bochniarz *et al.* 2013]. The upward trend for the percentage prevalence of CNS mastitis is probably world-wide [Thorberg *et al*. 2009, Sampimon *et al*. 2011]. As a result it is crucial to describe the role of CNS for both animal and human health.

The knowledge of how CNS *mastitis* develops, spreads in herds and persists is limited [Lam et al. 1997]. Also papers related to the risk to public health, originating from dairy cattle *mastitis* CNS are scarce [Hameed *et al*. 2006, Osman *et al*. 2015]. Bovine strains of CNS are poorly characterised; moreover, they are characterised mainly in terms of phenotypes. For these reasons, the aim of the paper is to summarise knowledge on the characterisation of CNS strains in the context of the risk to human and animal health.

Characteristics of CNS causing mastitis

CNS were traditionally perceived as bacteria that cause mild, subclinical inflammation and protect the udder against major pathogens [Waller *et al*. 2011]. In contrast, at present they are called "emerging *mastitis* pathogens" [Pyorala and Taponen 2009] and their role in the aetiology of animal diseases has been systematically increasing. Few factors characterise the course of CNS *mastitis*. The condition is usually mild, subclinical and sometimes self-curable. To our knowledge there is no information on the extent of pathogen eradication after recovery. It is likely that CNS species, present in the animal environment (skin), differ from those found in milk. Leroy *et al*. [2015] when comparing *S. haemolyticus* subtypes from milk and teat apices indicated that the intramammary infection causing agent likely originates from the teat skin. Usually only one species of CNS causes *mastitis*; however, Piessens *et al*. [2011] found two strains in milk from cows with *mastitis*.

At present the *staphylococci* group comprises 52 species and 28 subspecies [Parte 2014]. About forty species of CNS are known and may be distinguished using molecular methods. Not all of them, however, are typical of bovine *mastitis* [Waller *et al*. 2011]. The most frequently isolated bovine CNS include *S. chromogenes, S. xylosus, S. simulans, S. epidermidis, S. haemolyticus* and *S. sciuri* [Taponen and Pyorala 2009, Thorberg *et al*. 2009, Piessens *et al*. 2011, Waller *et al*. 2011, Frey *et al*. 2013, Schmidt *et al*. 2015]. *Staphylococcus xylosus* was found to be the most prevalent species among bovine CNS in Poland. Other main CNS species isolated

from milk of Polish cows included *S. chromogenes, S. sciuri* and *S. haemolyticus* [Malinowski *et al*. 2006, Bochniarz *et al*. 2013].

To date papers on different *mastitis*-causing CNS species have provided only a limited body of data, rarely confirmed by genetic information. It may be concluded that *S. chromogenes* infections respond better to antibiotics (possessing fewer antibiotic resistance genes?). On the other hand, *S. chromogenes* [Piessens *et al*. 2011] infections can be clinical in nature or persist in the udder. Aarestrup *et al*. [1999] found that also *S. simulans* infections can persist. Thorberg *et al*. [2009] noticed that *S. chromogenes* is a typical cause of *mastitis* in primiparous cows, while *S. epidermidis* is characteristic to older animals. Another fact is that most *S. epidermidis* strains lack genes coding for enterotoxin, leukocidin, protein A and α-toxin [Fey and Olson 2010]. In a study of Thorberg *et al*. [2009] the authors suspected that some *S. xylosus mastitis* cases were related with false positive milk samples. They found that the growth of *S. xylosus* in laboratory cultures may be connected with improper milk sampling or transport at excessively too high temperatures and even if the sampling procedure was appropriate, only teat canals could have been colonised with the bacteria, but not the udder itself.

Differences and similarity between CNS and *S. aureus mastitis*

It is not clear how to interpret differences and similarities between CNS and *S. aureus mastitis*. The statement of Pyorala and Taponen [2009] that CNS are not so different from *S. aureus* as *mastitis* aetiological agents is worth analysing. Typically *S. aureus* evokes a greater increase in somatic cell counts (SCC) than CNS, but the pattern for SCC (SCC being elevated for an extensive period) is similar. Moreover, the damage to the udder tissue is similar between CNS and *S. aureus*. What is very interesting and alarming, is the fact that CNS can persist in the udder in a way similar to *S. aureus* [von Eiff *et al*. 1999, Atalla *et al*. 2010] in the so-called small colony variant (SCV) phenotype.

CNS virulence factors that play a role during mastitis in dairy cattle

The development and outcome of bacterial infections depend on three main abilities of the pathogen: to invade the host (to penetrate through the barriers of skin and mucosal surfaces), to colonise the host and to successfully multiply inside the organism (and evade the following steps of immune response). Those traits are connected to the staphylococcal pathogenicity or virulence factors. The pathogenicity of *staphylococci* in the bovine udder is not fully understood. There are some problems with the definition of virulence factors, and, since the introduction of digital databases in the 1990's many proteins have been classified as virulence factors [Waasenaar and Gaastra 2001]. The functional classes of staphylococcal virulence factors may be divided into mediators of adhesion, protectors from the host's immune response

and substances that destroy the host's tissues. They may also be divided based on their origin into secreted proteins and structural components of the bacterial cell. The virulence factors are produced in relation to the growth phase of bacteria, *e.g.* microbial surface components recognising adhesive matrix molecules (MSCRAMMs) during the logarithmic growth, and toxins – during the stationary phase [Gordon and Lowy 2008]. For the purpose of the review paper the authors use all factors connected to virulence: "virulence factors" without distinguishing *e.g.*: true virulence factors, virulence associated factors, virulence life-style factors as defined by Waasenaar and Gaastra [2001]. Analysis of the literature references allows to distinguish from the factors that contribute to pathogenicity and virulence of the *staphylococci*, those that can be present in dairy cattle *mastitis* strains. Factors that will be discussed in this paper were selected subjectively by the authors based on their significance for public health (toxins, antimicrobial resistance) and their importance for animal health (forming of biofilm, evasion of phagocytosis, intracellular survival) and *mastitis* outcome. Description of the bacterial factors in some cases refers to *S. aureus* genes, since due to the inadequate recognition of CNS genetics, previous research was frequently conducted applying *S. aureus* specific primers. It is strongly suggested that the same or homologous factors are responsible for virulence in CNS. Another reason for such an approach is that *S. aureus* is normally used as a positive control for CNS virulence studies (and *S. carnosus* – as a negative). The paper description often refers to *S. epidermidis*, as *S. epidermidis* is probably the most recognised of CNS species due to its significance in human diseases, and research on its virulence factors is quite common. Where it is possible, the description refers to the *mastitis*-causing species.

Genetic determination of staphylococcal pathogenicity and virulence is complicated. In some virulence factors their expression undergoes constant changes. Virulence factors firstly were identified exclusively as constituents of pathogen genomes. However, it was found that they may be coded by genes of commensal bacteria as well, therefore skin *staphylococci* can be donors of virulence genes for *S. aureus*. Virulence factors may be coded in the core genome as well as the accessory genome in the form of genomic islands (formerly known as pathogenic islands and islets). Expression of such factors is regulated regarding environmental conditions by global regulators such as the staphylococcal accessory regulator (sar) and the accessory gene regulator (agr).

Microbial surface components recognising adhesive matrix molecules

MSCRAMMs take a crucial part in bacterial adhesion processes. They are associated to the bacterial cell wall surface and recognise macromolecular ligands of the host's extracellular matrix. Fibronectin-binding proteins are of particular importance during staphylococcal infection, as stated by Sinha *et al*. [2000]. Many of the adhesion factors are encoded both in *S. aureus* and in CNS genomes. The *S. epidermidis* surface proteins (ses) A, E, G, H, I, C, coded by genes *sesA, sesE, sesG,*

sesH, sesI, sesC, and autolysin/adhesin proteins, coded by *aae* and *atlE* genes, belong to the main CNS adhesive matrix molecules [Fey and Olson 2010]. Other proteins comprising MSCRAMMs include the serine-aspartate repeat (coded by *sdrG* gene), helping bacteria to adhere to fibrinogen and extracellular matrix-binding protein (coded by the *Embp* gene), sufficient and necessary for biofilm formation [Christner *et al*. 2010].

Biofilm formation

Biofilm production is strongly connected to pathogenicity of bacteria. As suggested e.g. by Krukowski *et al*. [2008], bovine *mastitis* strains of *S. aureus* exhibit a considerable potential to produce biofilm (slime). At the same time, CNS are believed to produce biofilm as one of their main virulence factors, allowing them to adhere to the host's tissue. The functions of slime, apart from facilitation of the host colonisation, include impairment of phagocytosis and protection from antimicrobial drugs. Simojoki *et al*. in 2011 found that *S. epidermidis* is a main biofilm-producing species among bovine CNS, which probably contributes to the persistence of udder infections caused by these bacteria. *Staphylococcus epidermidis* is also the main cause of human device-related infections, with biofilm being a crucial (or according to some authors even exclusive) virulence factor of this species. The intracellular adhesion gene *(ica)* is connected to slime production in bacteria. Genes localised in the *ica* operon code for enzymes are involved in the synthesis of polysaccharide intracellular protein, the main staphylococcal biofilm component. For biofilm production an expression of genes *icaA* and *icaB* is essential [Krukowski *et al.* 2008]. The *agr* system controls quorum sensing, a phenomenon connected to biofilm formation and functioning. In *S. aureus* (and in CNS) the *agr* operon is connected to the expression of multiple virulence genes and the suppression of the *agr* promoter by protein sarX (coded by *sarX* gene) results in the suppression of biofilm production [Rowe *et al*. 2011]. Wang *et al*. [2008] found that the main factor regulating biofilm synthesis (and *S. epidermidis* virulence in general) is sarZ – one of the global regulators – (coded by *sarZ* gene). Other genes involved in biofilm formation include the fibrinogen binding protein gene (*fbe),* the accumulation associated protein gene *(aap), atlE,, and Embp* [Schommer *et al*. 2011, Gill *et al*. 2005] – the two latter ones were mentioned earlier in the "MSCRAMMs" section.

Factors that facilitate evasion of phagocytosis

There are many factors that facilitate evasion of phagocytosis. About 60% of human *S. aureus* strains [Foster 2005] developed proteins called chemotaxis inhibitory proteins that prevent neutrophils from migration to the infection site. Apart from chemotaxis inhibitory proteins, these bacteria express more means of preventing the neutrophil action. CNS, e.g. *S. aureus*, can evade phagocytosis. Avall-Jaaskelainen

et al. [2013] found that only *S. chromogenes* is fully susceptible to phagocytosis. Among the other analysed CNS species, *S. simulans* and *S. agnetis*, on average 40% of the bacteria resisted phagocytosis, with the emphasis given by authors to *S. simulans*. Antimicrobial peptide sensors (aps), coded by genes *apsX, apsR, apsS, graR* and *graS*, are secreted by *staphylococci* to avoid the influence of the neutrophil antimicrobial proteins [Fey and Olson 2010, Otto 2013]. After the bacteria are engulfed by phagocytes, they provoke formation of the extra space in phagosomes, allowing them to survive inside.

Capsule production

Capsule production is an important factor facilitating evasion of phagocytosis. Encapsulated bacteria are more resistant to phagocytosis than "bare" pathogens. *Staphylococcus epidermidis* possess a unique virulence factor – the poly-γ-DL-glutamic acid capsule, coded by genes *capA, capB, capC* and *capD* [Fey and Olson 2010]. Gill *et al*. [2005] stated that the *cap* operon was transmitted via horizontal gene transfer from *Bacillus anthracis* during the species evolution. Hybridisation experiments undertaken by Tollersrud *et al*. [2000] showed that bovine *S. haemolyticus* possesses genes involved in serotype 5 capsule production. On the other hand, none of the analysed *S. xylosus*, *S. chromogenes*, *S. simulans* or *S. intermedius* strains had such genes.

Enterotoxins

Enterotoxins produced by *staphylococci* are heat-stable, which makes them a serious threat to human health. Pasteurization destroys live bacterial cells, but if the enterotoxins were present in raw milk, they are likely to be present in pasteurized milk as well [Hameed *et al*. 2006]. They are also resistant to such conditions as freezing and drying and not susceptible to enzymatic digestion in the human gastrointestinal tract [Kadariya *et al*. 2014]. Staphylococcal enterotoxins (SE) produced both by *S. aureus* and CNS belong to the family of pyrogenic toxins (along with Toxic Shock Syndrome Toxin-1 (TSST-1). They are a common cause of food poisonings. The information on enterotoxin production by CNS is scarce, but clear: these bacteria are able to produce SE, predominantly SEA, SED, SEE and SEH [Zell *et al*. 2008]. In their latest study Fijalkowski *et al*. [2014] found enterotoxin or TSST-1 genes (*tst-1*) in 53.3% of *S. xylosus* strains. Genes coding for SE are present both in chromosomal DNA and in mobile genetic elements, e.g. pathogenicity islands, phages (*sea, sep*), transposons and plasmids (*sec1, sed, sej*). Enterotoxin genes are localised in the *agr* region. The enterotoxin gene cluster *(egc)* operon with several genes coding for toxins (seg, sei, sem, seo) is localised inside the *agr* region. The same region is responsible for the above-mentioned biofilm production.

Leukotoxins, α- and β- haemolysins and protease production

Leukotoxins have the capacity of selectively killing phagocytic cells. Neutrophils and macrophages are essential for the innate immune response. Staphylococcal infection outcome largely depends on the neutrophil function. The staphylococcal leukotoxin family consists of leukotoxins and γ-haemolysin. Information on leukotoxin-producing bovine CNS is scarce. It was proven by Burriel and Dagnall [1997] based on bovine *mastitis* CNS that bacteria producing leucotoxins are capable of killing 50% of polymorphonuclear leukocytes isolated from bovine mammary gland. Among leukotoxins (Luk) secreted by *S. aureus*, the most important include LukM (coded by the *LukM* gene) – highly active against bovine polymorphonuclear leukocytes [Rainard *et al*. 2003] and Panton-Valentine Leucocidin (PVL). It is known that CNS are able to produce PVL coded by the *pvl* gene [Unal and Cinar 2012].

Haemolysins are among the most widely recognised staphylococcal virulence factors. Staphylococcal α-toxin (α-haemolysin) is coded in the bacterial genome by the *hla* gene and is produced by some CNS species. Bochniarz *et al*. [2013] reported only α-haemolytic (not β-haemolytic) activity in 21% of the Polish CNS strains isolated from cow's milk. These bacteria belonged to the *S. haemolyticus* species. On the other hand, Zell *et al*. [2008] found that CNS exhibit mostly β-haemolytic activity (although among analysed species the only one clearly connected to bovine *mastitis* was *S. xylosus*). Wang *et al*. [2008] suspected that *sarZ* – one of the global regulators is responsible for haemolytic activity in *S. epidermidis*.

CNS are able to produce proteases. Bochniarz *et al*. [2013] reported proteolytic activity of *S. chromogenes* and *S. sciuri* isolates from bovine *mastitis*. Phenol-soluble modulins are a relatively new class of *agr* regulated cytolytic toxins. They are linked to opportunistic diseases in humans [Nunes Botelho *et al*. 2012]. Phenol-soluble modulins are coded by the *beta1, beta2, hld, alfa* and *delta* genes and they are produced by *S. epidermidis* [Gill *et al*. 2005]. They have not been characterised in the bovine CNS strains.

Transmission between humans and dairy cows

It is not common for the staphylococcal *mastitis* strains to be transmitted between humans and dairy cows. However, it was reported [Sakwinska *et al*. 2011] that such a transmission is possible. Moreover, Thorberg *et al*. in 2006 found the same strains of CNS (*S. epidermidis*) on milkers' hands and in milk and suspected that workers are the source of bacteria to cows. CNS often accompanies infections with major pathogens that are treated with antimicrobial drugs (the author's study). The idea introduced e.g. by Frey *et al*. [2013] and Otto [2013] that CNS harbour drug resistance genes for other bacteria, such as *S. aureus*, *Streptococcus* species or *E. coli*, is very disturbing.

Mechanisms to avoid the action of antibiotics

Bacteria created several mechanisms to avoid the action of antibiotics. Many multidrug resistant and even extremely resistant strains have evolved since the introduction of antimicrobial drugs. Bacteria exploit several mechanisms to avoid antimicrobial action. They produce enzymes (e.g. β-lactamase) that break down drug compounds. They cause an efflux of the active substance from the bacterial cell to the periplasmatic space with the use of the specific pump (regarding tetracycline in many Gram-negative and Gram-positive bacteria). Another mechanism that pathogens developed to face the drug challenge is the modification of the antibiotic molecule. The change in the molecule makes drugs ineffective. Bypassing the drug-blocked metabolic trace is another way, by which bacteria gain resistance to antibiotics (e.g. sulphonamides). Probably the most widely recognised drug resistance in *staphylococci* is methicillin-resistance. Among human strains of methicillin-resistant *Staphylococcus aureus* (MRSA), both hospital acquired (HA-MRSA) and community acquired (CA-MRSA), β-lactam resistance is extremely popular. MRSA are not as common among *mastitis* strains, but 27 strains of MRSA were isolated from cow's milk between 2002 and 2004 in Hungary. Juhasz-Kaszanyitzky *et al*. [2007] suspected that analysed bacteria can be transmitted between humans and animals, therefore they pose a serious threat to human health. β-lactamase production is the most common factor responsible for resistance to penicillin. The enzyme is coded by the *blaZ* gene. Another mechanism is binding of penicillin by the product of the *mecA* gene – penicillin-binding protein 2a. A study on Swedish CNS strains showed that β-lactamase production is most common in *S. epidermidis* and *S. haemolyticus* species (41% of studied strains) [Waller *et al*. 2011]. Barbier *et al*. [2010], suggested that *S. epidermidis* may be a source of methicillin resistance genes for *S. aureus* in the case of CA-MRSA. CNS are more often multi-drug resistant than *S. aureus* and generally responds weakly to the treatment. Frey *et al*. [2013] concluded that, among 417 bovine strains of different (19 species) CNS, 47% were resistant to oxacillin, 33.8% to fusidic acid, 31.9% to tiamulin, 23.3% to penicillin and 15.8% to tetracycline. A total of 15.1% isolates were resistant to 2 or more antibiotics [Frey *et al.* 2013]. Oxacillin resistance genes (*blaZ, mecA*) are present in 70% to 80% of the CNS strains [Strommenger *et al*. 2003, Frey *et al.* 2013]. Tetracyclines, which inhibit bacterial protein synthesis via interaction with RNA, are among the most widely used antimicrobials. There are several dozen *Tet* genes (*tetK, tetM*) – that encode efflux pumps [Strommenger *et al*. 2003]. Other genes, such as *Nor (NorA, NorB, NorC), MepA, MdeA, SepA, SdrM, LmrS,* among which *NorA* is the most popular, code for efflux pumps [Costa *et al*. 2013]. Erythromycin resistance genes may surely be present in CNS genomes [Strommenger *et al*. 2003]. Streptomycin resistance is coded by the *str* and *nt6-l* genes [Frey *et al*. 2013].

Intracellular survival

It was noticed by Taponen *et al.* [2007] that infections caused by CNS persist. Bacteria survived to the first lactation inside the udders of pregnant heifers. Bacterial survival inside the host's cells is frequently associated with the formation of the so-called small colony variants (SCV). The small colony variant is a bacterial phenotype, which in the opinion of some authors originates from stable genetic mutations [Sendi and Proctor 2009]. According to other researchers SCVs evade the so-called phenotype switching after e.g. prolonged exposure to antibiotics [Pawlik *et al*. 2012]. *Staphylococcus aureus* SCVs in dairy cows are a cause of chronic, persistent *mastitis* and are probably more common than previously thought [Atalla *et al*. 2010]. CNS, such as e.g. *S. aureus* can form the SCV phenotype as it was proven by Almeida and Oliver [2001] and von Eiff *et al*. [1999], and survive inside macrophages, neutrophils and mammary epithelial cells [Almeida and Oliver 2001]. Invasion of mammary epithelial cells by SCVs includes bacterial adherence to the epithelial cell surface, formation of pseudopod-like structures and engulfment within endocytic vesicles. Toxic SCV strains then secrete the pore-forming α-toxin and escape to the cytoplasm. Bacterial leucocidins are secreted to evade lysosomal destruction [Atalla *et al*. 2010]. Intracellular survival of *S. epidermidis* is likely to be connected to its biofilm production ability, but the SCV problem has not been examined regarding CNS in bovine *mastitis*. The *S. aureus* SCV formation is connected to changes in the expression of the global regulator *agr* and mutations in genes coding for the electron transport chain proteins [Proctor *et al*. 2014]. Mitchell *et al*. [2013] found that *SigB* is the main regulator of virulence in *S. aureus* small colony variants. According to those authors, the virulence-associated gene expression profile of SCVs differs from that of prototypical strains and is often influenced by *SigB* rather than by the *agr* system.

Conclusions

The pathological role of CNS in bovine *mastitis* has increased inevitably and there is an urgent need to highlight the threat to both human and animal health, originating from CNS. There are many genes engaged in CNS pathogenicity and virulence during dairy cattle mastitis. MSCRAMMs are one of the factors that contribute to bacterial adhesion processes. The main CNS adhesive matrix molecules are coded by a group of the *ses* genes and by the *aae*, *atlE, sdrG* and *Embp* genes [Christner *et al*. 2010, Fey and Olson 2010]. Genes localised in the *ica* and *agr* operon and sarZ, a global regulator coded by the *sarZ* gene are the main genes regulating staphylococcal biofilm synthesis and biofilm components [Krukowski *et al.* 2008, Wang *et al*. 2008, Rowe *et al*. 2011]. Also other genes, e.g. *fbe*, *aap, atlE, and Embp*, are involved in biofilm formation [Gill *et al*. 2005, Schommer *et al*. 2011]. The importance of CNS in bovine *mastitis* depends on factors that facilitate evasion of phagocytosis. *Staphylococci,* to avoid the influence of the neutrophil antimicrobial proteins, produce antimicrobial

peptide sensors (aps) coded by the *aps* and *gra* genes [Fey and Olson 2010, Otto 2013]. Also capsule production coded by the *cap* genes [Fey and Olson 2010] is an important factor facilitating evasion of phagocytosis; however, not all CNS strains possess this unique virulence factor. A serious threat to human health is posed by enterotoxins produced by *staphylococci,* because these toxins are heat-stable. Genes coding for enterotoxins are mainly localised in the same region, which is responsible for biofilm production [Fijalkowski *et al*. 2014]. Leukotoxins have the capacity of selectively killing phagocytic cells. Their production is coded by the *LukM* and *pvl* genes [Rainard *et al*. 2003, Unal and Cinar 2012]. Staphylococcal α-haemolysin is produced by some of the CNS species causing mastitis and is coded by the *hla* gene [Bochniarz *et al*. 2013]. According to other authors, CNS exhibit mostly the β-haemolytic activity [Zell *et al*. 2008]. In *S. epidermidis sarZ* - one of the global regulators, is responsible for the haemolytic activity [Wang *et al*. 2008]. CNS are also able to produce proteases, but they have not been characterised in the bovine CNS strains. CNS created several mechanisms to avoid the action of antibiotics, which are coded by the *blaZ, mecA* genes (resistance to penicillin, oxacillin), the *tet, Nor, MepA, MdeA, SepA, SdrM, LmrS* genes (resistance to tetracyclines), the *erm* gene (resistance to erythromycin) and *str*, *nt6-l* (resistance to streptomycin). CNS are often multidrug resistant and in general respond weakly to the treatment. Infections caused by CNS can persist over an extensive period of time inside the udders, causing chronic, persistent mastitis [Taponen *et al.* 2007]. CNS can form the SCV phenotype, as it was proven by Almeida and Oliver [2001] and von Eiff *et al*. [1999], and survive inside macrophages, neutrophils and mammary epithelial cells [Almeida and Oliver 2001]. To date the SCV problem has not been examined regarding CNS in bovine *mastitis*.

REFERENCES

1. AARESTRUP F.M., LARSEN H.D., JENSEN N.E., 1999 – Characterization of Staphylococcus simulans strains isolated from cases of bovine mastitis. ***Veterinary Microbiology*** 66, 165-170.
2. ALMEIDA R.A., OLIVER S.P., 2001 – Interaction of coagulase-negative Staphylococcus species with bovine mammary epithelial cells. ***Microbial Pathogenesis*** 31, 205-212.
3. ATALLA H., GYLES C., MALLARD B., 2010 – Persistence of a Staphylococcus aureus small colony variants (S-aureus SCV) within bovine mammary epithelial cells. ***Veterinary Microbiology*** 143, 319-328.
4. AVALL-JAASKELAINEN S., KOORT J., SIMOJOKI H., TAPONEN S., 2013 – Bovine-associated CNS species resist phagocytosis differently. ***BMC Veterinary Research*** 9.
5. BARBIER F., RUPPE E., HERNANDEZ D., LEBEAUX D., FRANCOIS P., FELIX B., DESPREZ A., MAIGA A., WOERTHER P-L., GAILLARD K., JEANROT C., WOLFF M., SCHRENZEL J., ANDREMONT A., RUIMY R., 2010 – Methicillin-Resistant Coagulase-Negative Staphylococci in the Community: High Homology of SCCmec IVa between Staphylococcus epidermidis and Major Clones of Methicillin-Resistant Staphylococcus aureus. ***Journal of Infectious Diseases*** 202, 270-281.
6. BOCHNIARZ M., WAWRON W., SZCZUBIAL M., 2013 – Coagulase-negative staphylococci (CNS) as an aetiological factor of mastitis in cows. ***Polish Journal of Veterinary Sciences*** 16, 487-492.

7. BURRIEL A.R., DAGNALL G.J.R., 1997 – Leukotoxic factors produced by staphylococci of ovine origin. ***Microbiological Research*** 152, 247-250.
8. CHRISTNER M., FRANKE G.C., SCHOMMER N.N., WENDT U., WEGERT K., PEHLE P., KROLL G., SCHULZE C., BUCK F., MACK D., AEPFELBACHER M., ROHDE H., 2010 – The giant extracellular matrix-binding protein of Staphylococcus epidermidis mediates biofilm accumulation and attachment to fibronectin. ***Molecular Microbiology*** 75, 187-207.
9. COSTA S.S., VIVEIROS M., AMARAL L., COUTO I., 2013 – Multidrug Efflux Pumps in Staphylococcus aureus: an Update. ***The Open Microbiology Journal*** 7, 59-71.
10. FEY P.D., OLSON M.E., 2010 – Current concepts in biofilm formation of Staphylococcus epidermidis. ***Future Microbiology*** 5, 917-933.
11. FIJALKOWSKI K., STRUK M., KARAKULSKA J., PASZKOWSKA A., GIEDRYS-KALEMBA S., MASIUK H., CZERNOMYSY-FUROWICZ D., NAWROTEK P. 2014 – Comparative Analysis of Superantigen Genes in Staphylococcus xylosus and Staphylococcus aureus Isolates Collected from a Single Mammary Quarter of Cows with Mastitis. Journal of Microbiology 52, 366-372.
12. FOSTER T. J., 2005 – Immune evasion by Staphylococci. ***Nature Reviews Microbiology*** 3, 948-958.
13. FREY Y., RODRIGUEZ J.P., THOMANN A., SCHWENDENER S., PERRETEN V., 2013 – Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis milk. ***Journal of Dairy Science*** 96, 2247-2257.
14. GILL S.R., FOUTS D.E., ARCHER G.L., MONGODIN E.F., DEBOY R.T., RAVEL J., PAULSEN I.T., KOLONAY J.F., BRINKAC L., BEANAN M., DODSON R.J., DAUGHERTY S.C., MADUPU R., ANGIUOLI S.V., DURKIN A.S., HAFT D.H., VAMATHEVAN J., KHOURI H., UTTERBACK T., LEE C., DIMITROV G., JIANG L.X., QIN H.Y., WEIDMAN J., TRAN K., KANG K., HANCE I.R., NELSON K.E., FRASER C.M., 2005 – Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant Staphylococcus aureus strain and a biofilm-producing methicillin-resistant Staphylococcus epidermidis strain. ***Journal of Bacteriology*** 187, 2426-2438.
15. GORDON R.J., LOWY F.D., 2008 – Pathogenesis of methicillin-resistant Staphylococcus aureus infection. ***Clinical Infectious Diseases*** 46, S350-S359.
16. HAMEED K.G.A., SENDER G., KORWIN-KOSSAKOWSKA A., 2007 – Public health hazard due to mastitis in dairy cows. ***Animal Science Papers and Reports*** 25, 73-85.
17. JUHASZ-KASZANYITZKY E., JANOSI S., SOMOGYI P., DAN A., VAN DER GRAAF-VAN BLOOIS L., VAN DULIJKEREN E., WAGENAAR J.A., 2007 – MRSA transmission between cows and humans. ***Emerging Infectious Diseases*** 13, 630-632.
18. KADARIYA J., SMITH T.C., THAPALIYA D., 2014 – Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. ***BioMed Research International*** 2014, 827965-827965.
19. KRUKOWSKI H., SZYMANKIEWICZ M., LISOWSKI A., 2008 – Slime production by Staphylococcus aureus strains isolated from cases of bovine mastitis. ***Polish Journal of Microbiology*** 57, 253-255.
20. LEROY F., VAN COILLIE E., BRAEM G., PIESSENS V., VERBIST B., DE VUYST L., DE VLIEGHER S., 2015 – Short communication: Subtyping of Staphylococcus haemolyticus isolates from milk and corresponding teat apices to verify the potential teat-skin origin of intramammary infections in dairy cows. ***Journal of Dairy Science*** 98, 7893-7898.
21. LAM T.J.G.M., VANVLIET J.H., SCHUKKEN, Y.H., GROMMERS F.J., VANVELDENRUSSCHER A., BARKEMA H.W., BRAND A. 1997 – The effect of discontinuation of postmilking teat disinfection in low somatic cell count herds 2. Dynamics of intramammary infections. ***Veterinary Quarterly*** 2, 47-53.

22. MALINOWSKI E., LASSA H., KLOSSOWSKA A., SMULSKI S., MARKIEWICZ H., KACZMAROWSKI A., 2006 – Etiological agents of dairy cows' mastitis in western part of Poland. ***Polish Journal of Veterinary Sciences*** 9, 191-194.
23. MITCHELL G., FUGERE A., GAUDREAU K.P., BROUILLETTE E., FROST E.H., CANTIN A.M., MALOUIN F., 2013 – SigB Is a Dominant Regulator of Virulence in Staphylococcus aureus Small-Colony Variants. ***Plos One*** 8.
24. NUNES BOTELHO A.M., NUNES Zd. G., ASENSI M.D., RIBEIRO GOMES M.Z., LONGO FRACALANZZA S.E., SA FIGUEIREDO A.M., 2012 – Characterization of coagulase-negative staphylococci isolated from hospital indoor air and a comparative analysis between airborne and inpatient isolates of Staphylococcus epidermidis. ***Journal of Medical Microbiology*** 61, 1136-1145.
25. OSMAN K.M., ABD EL-RAZIK K.A., MARIE H.S.H., ARAFA A. 2015 – Relevance of biofilm formation and virulence of different species of coagulase-negative staphylococci to public health. ***European Journal of Clinical Microbiology & Infectious Diseases*** 34, 2009-2016.
26. OTTO M., 2013 – Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection. ***Bioessays*** 35, 4-11.
27. PARTE A.C., 2014 – LPSN-list of prokaryotic names with standing in nomenclature. ***Nucleic Acids Research*** 42, D613-D616.
28. PAWLIK A., SENDER G., STARZYNSKI R., KORWIN-KOSSAKOWSKA A., 2012 – Genetic basis of host innate immune response in mastitis caused by Staphylococcus aureus. ***Central European Journal of Immunology*** 37, 405-409.
29. PIESSENS V., VAN COILLIE E., VERBIST B., SUPRE K., BRAEM G., VAN NUFFEL A., DE VUYST L., HEYNDRICKX M., DE VLIEGHER S., 2011 – Distribution of coagulase-negative Staphylococcus species from milk and environment of dairy cows differs between herds. ***Journal of Dairy Science*** 94, 2933-2944.
30. PROCTOR R.A., KRIEGESKORTE A., KAHL B.C., BECKER K., LOFFLER B., PETERS G., 2014 – Staphylococcus aureus Small Colony Variants (SCVs): a road map for the metabolic pathways involved in persistent infections***. Frontiers in Cellular and Infection Microbiology*** 4.
31. PYORALA S., TAPONEN S., 2009 – Coagulase-negative staphylococci - Emerging mastitis pathogens. ***Veterinary Microbiology*** 134, 3-8.
32. RAINARD P., CORRALES J.C., BARRIO A.B., COCHARD T., POUTREL B., 2003 – Leukotoxic activities of Staphylococcus aureus strains isolated from cows, ewes, and goats with mastitis: Importance of LukM/LukF '-PV leukotoxin. ***Clinical and Diagnostic Laboratory Immunology*** 10, 272-277.
33. ROWE S.E., MAHON V., SMITH S.G., O'GARA J.P., 2011 – A novel role for SarX in Staphylococcus epidermidis biofilm regulation. ***Microbiology-Sgm*** 157, 1042-1049.
34. SAKWINSKA O., GIDDEY M., MOREILLON M., MORISSET D., WALDVOGEL A., MOREILLON P., 2011. Staphylococcus aureus Host Range and Human-Bovine Host Shift. ***Applied and Environmental Microbiology*** 77, 5908-5915.
35. SAMPIMON O.C., LAM T.J.G.M., MEVIUS D.J., SCHUKKEN Y.H., ZADOKS R.N., 2011 – Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples. ***Veterinary Microbiology*** 150, 173-179.
36. SCHMIDT T., KOCK M.M., EHLERS M.M., 2015 – Diversity and antimicrobial susceptibility profiling of staphylococci isolated from bovine mastitis cases and close human contacts. ***Journal of Dairy Scien***ce 98, 6256-6269.
37. SCHOMMER N.N., CHRISTNER M., HENTSCHKE M., RUCKDESCHEL K., AEPFELBACHER M., ROHDE H., 2011 – Staphylococcus epidermidis Uses Distinct Mechanisms of Biofilm Formation to Interfere with Phagocytosis and Activation of Mouse Macrophage-Like Cells 774A.1. ***Infection and Immunity*** 79, 2267-2276.

38. SENDER G., KORWIN-KOSSAKOWSKA A., HAMEID K.G.A., PRUSAK B., 2006 – Association of the polymorphism of some genes with the occurrence of mastitis in cattle. ***Medycyna Weterynaryjna*** 62, 5, 563-565.
39. SENDER G., KORWIN-KOSSAKOWSKA A., STEPINSKA U., 2003 – Utilizing genetic markers for mastitis resistance ***Medycyna Weterynaryjna*** 59,10, 853-856.
40. SENDI P., PROCTOR R.A., 2009 – Staphylococcus aureus as an intracellular pathogen: the role of small colony variants. ***Trends in Microbiology*** 17, 54-58.
41. SIMOJOKI H., SALOMAKI T., TAPONEN S., IIVANAINEN A., PYORALA S. 2011 – Innate immune response in experimentally induced bovine intramammary infection with Staphylococcus simulans and S. epidermidis. ***Veterinary Research*** 42.
42. SINHA B., HERRMANN M., KRAUSE K.H., 2000 – Is Staphylococcus aureus an intracellular pathogen? Response. ***Trends in Microbiology*** 8, 343-344.
43. STROMMENGER B., KETTLITZ C., WERNER G., WITTE W., 2003 – Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. ***Journal of Clinical Microbiology*** 41, 4089-4094.
44. TAPONEN S., KOORT J., BJORKROTH J., SALONIEMI H., PYORALA S., 2007 – Bovine intramammary infections caused by coagulase-negative staphylococci may persist throughout lactation according to amplified fragment length polymorphism-based analysis. ***Journal of Dairy Science*** 90, 3301-3307.
45. TAPONEN S., PYORALA S., 2009 – Coagulase-negative staphylococci as cause of bovine mastitis - Not so different from Staphylococcus aureus? ***Veterinary Microbiology*** 134, 29-36.
46. THORBERG B.M., DANIELSSON-THAM M.L., EMANUELSON U., WALLER K.P. 2009 – Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. ***Journal of Dairy Science*** 92, 4962-4970.
47. THORBERG B.M., KUHN I., AARESTRUP F.M., BRANDSTROM B., JONSSON P., DANIELSSON-THARN M.L., 2006 – Pheno- and genotyping of Staphylococcus epidermidis isolated from bovine milk and human skin. ***Veterinary Microbiology*** 115, 163-172.
48. TOLLERSRUD T., KENNY K., REITZ A.J., LEE J.C., 2000. Genetic and serologic evaluation of capsule production by bovine mammary isolates of Staphylococcus aureus and other Staphylococcus spp. from Europe and the United States. ***Journal of Clinical Microbiology*** 38, 2998-3003.
49. UNAL N., CINAR O.D., 2012 – Detection of staphylococcal enterotoxin, methicillin-resistant and Panton-Valentine leukocidin genes in coagulase-negative staphylococci isolated from cows and ewes with subclinical mastitis. ***Tropical Animal Health and Production*** 44, 369-375.
50. VON EIFF C., VAUDAUX P., KAHL B.C., LEW D., EMLER S., SCHMIDT A., PETERS G., PROCTOR R.A., 1999 – Bloodstream infections caused by small-colony variants of coagulase-negative staphylococci following pacemaker implantation. ***Clinical Infectious Diseases*** 29, 932-934.
51. WASSENAAR T.M., GAASTRA, W., 2001 – Bacterial virulence: can we draw the line? ***Fems Microbiology Letters*** 201, 1-7.
52. WALLER K.P., ASPAN A., NYMAN A., PERSSON Y., ANDERSSON U.G., 2011 – CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis. ***Veterinary Microbiology*** 152, 112-116.
53. WANG L., LI M., DONG D., BACH T-H.L., STURDEVANT D.E., VUONG C., OTTO M., GAO Q. 2008 – SarZ is a key regulator of biofilm formation and virulence in Staphylococcus epidermidis. ***Journal of Infectious Diseases*** 197, 1254-1262.
54. ZELL C., RESCH M., ROSENSTEIN R., ALBRECHT T., HERTEL C., GOETZ F., 2008 – Characterization of toxin production of coagulase-negative staphylococci isolated from food and starter cultures. ***International Journal of Food Microbiology*** 127, 246-251.