POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 35(2): 233–247, Y. 2020

OCCURRENCE AND CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM COWS WITH MASTITIS

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Key words: *Staphylococcus aureus*, mastitis, phenotyping, genotyping, biotyping.

Abstract

Mastitis is the most common disease of dairy cows, which causes significant economic losses in dairy farming. Mastitis caused by *Staphylococcus aureus* is particularly difficult to treat and eradicate, which is why rapid identification and evaluation of the pathogen and strain is important. The study presents occurrence and characterisation of *S. aureus* in the North – East region of Poland, and especially in the Podlaskie Voivodeship. Of the 9,617 samples of milk from cows suspected of mastitis, *S. aureus* was isolated from 1,330 samples, representing 19.4%. 225 strains of *S. aureus* were biotyped according to Umeki and classified into three biotypes: I – 19 (8.45%), III – 115 (51.11%), IV – 91 (40.44%). Biotype IV strains showed the most pathogenic characteristics. Randomly selected *S. aureus* strains were genotyped using ADSRRS-fingerprinting, this method was a better tool for differentiating strains compared to biotyping.

Introduction

Mastitis is the most common dairy cows' disease and contributes to significant economic losses in dairy farming. A particular problem is the inflammation caused by staphylococci, especially *Staphylococcus aureus*. This is due to the specific properties of *S. aureus*, among others: the ability to adhere to mammary gland cells, which facilitates tissue colonization (FROST 1975, AGUILAR et al. 2001), the ability to produce enzymes and toxins, which in turn enables the spread of bacteria in the mammary gland, while the polysaccharide capsular and coagulase impair immune

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defense (SORDELLI et al. 2000, SZYMAŃSKA and BUCZEK 1999). Slime and biofilm production enables staphylococci to survive in the mammary gland (VASUNDEVAN et. al. 2003, FOX et al. 2005, MELHIOR et al. 2006). In addition, the production of β-lactamase and the presence of the PBP2a protein reduces the sensitivity of these bacteria to β-lactam antibiotics, including methicillin (ŁOPACIUK and DZIERŻANOWSKA 2002). This results in mastitis which is difficult to treat and combat. In preventing the occurrence of new infections, it is important to quickly recognize and thoroughly understand the etiological factor. Effective typing of bacteria, especially staphylococci shows the pattern of transmission of infection, enables the identification of a reservoir of microorganisms and helps to eliminate existing infections and prevents the formation of new ones (MIĘDZYBRODZKI et al. 2008). Many phenotypic and genotypic methods are used to differentiate *S. aureus* strains isolated from cow's mammary gland. Phenotyping is the classification of strains based on the phenotypic characteristics of microorganisms. The phenotyping of *S. aureus* strains isolated from cow's milk uses: biotyping, i.e. the classification of bacteria based on specific biochemical characteristics (HAJEK and MARSALEK 1961, DEVRIESE et al. 1984, HAKIMI et al. 2016), serotyping (TOLLERSRUD et al. 2000, GUIDRY et al. 1998, SUTRA 1990, POUTREL 1988, BARDIAU et al. 2014, AMBROGGIO et al. 2018), phagotyping (MACKIE et al. 1987, AARESTRUP et al. 1997, FOX et al. 1991, LARSEN et al. 2000, VINTOV et al. 2003). Methods based on electrophoretic techniques are also used to analyze cellular protein profiles such as immunoblotting (LEITNER et al. 2003, YOUNIS et al. 2002) and MLEE (Multilocus Enzyme Electrophoresis) (FITZGERALD et al. 1997, TOLLERSRUD et al. 2000). One of the phenotypic methods for typing staphylococci is testing the antibiotic resistance profile (AARESTRUP et al. 1995, RAIMUNDO at al. 1999, LARSEN et al. 2000, VINTOV et al., 2003, ANDERSON et al. 2006, JAGIELSKI et al. 2014). It is a method commonly employed because of routine use of antibiograms, simplicity and low costs of testing. Commercial tests such as API 20 Staph system (bioMerieux), Api ID 32 Staph (bioMerieux), Staph-Zym (Rosco), the VITEK system (bioMerieux), Microgen Staph ID (Microgen Bioproducts) and STAPHYtest (PLIVA-Lachema) are commonly used to identify and type *S. aureus* strains (LISOWSKA-ŁYSIAK et al. 2018). Currently, genotypic methods are used for detection, identification, typing (differentiation) as well as in taxonomic studies or phylogenesis of *S. aureus*. In the study of genetic variation, molecular biology techniques are used, including chromosome size analysis, restricted digestion of the genome or plasmids and electrophoretic separation of its products. Analysis of plasmid profiles of *S. aureus* strains was one of the first methods used in research, but due to high variability and low

differentiating potential it is rarely used nowadays in epidemiological studies (AARESRUP et al. 1995, MATTHEWS et al. 1993, NASCIMENTO et al. 2005). REA-PFGE (Restriction Enzyme Analysis with Pulsed Field Gel Electrophoresis) is the "gold standard" in staphylococcus typing and is useful in epidemiological studies, detection of infection sources and in the case of infections caused by one strain (SABAT et al. 2013, LUNDBERG et al. 2016, JAGIELSKI et al. 2014, HATA et al. 2016, KOT et al. 2012, CASTELANI et al. 2013). The PCR (Polymerase Chain Reaction) technique that mimics the phenomenon of DNA replication is one of the most popular research techniques used to detect, identify, and determine phylogenetic relationships. Due to the advantage of sensitivity and speed of analysis, it is used in laboratories in various forms. For typing of *S. aureus* strains isolated from cow mastitis amplification of known genome regions combined with PCR/RFLP restriction analysis (LANGE et al. 1999) is used where strains are most often differentiated based on the amplification for example: coagulase (*coa*) gene fragments (AARESTRUP et al. 1995, DE SILVA and DE SILVA 2005, KARAKULSKA et al. 2011) and protein A (*spa*) (ANNEMÜLLER et al. 1999, LANGE et al. 1999, KUŹMA et al. 2005, HATA et al. 2016), genes encoding biofilm formation protein e.g. *ica* gene or *bap* gene (VASUNDEVAN et. al. 2003, SZWEDA et al. 2012, SALIMENA et al. 2016), genes encoding the ability to produce toxins (HAYAKAWA et al. 2001, LARSEN et al. 2002) or antibiotic resistance genes including methicillin resistance – the gene *mec*.

Next typing method is Random Amplified Polymorphic DNA amplifications (RAPD) – PCRs used to quickly differentiate *S. aureus* strains (LAM et al. 1996, SACHANOWICZ et al. 2007, NAWROTEK et al. 2009). Another variation of the PCR technique is the amplification of two or more different DNA fragments, i.e. Triplex PCR (SABAT et al. 2006, JAGIELSKI et al. 2014), Multiplex PCR (PUACZ et al. 2015). Ribotyping is based on differentiation of genes encoding the chromosomal RNA (rRNA) of the small and large ribosome subunits. The PCR (AARESTRUP et al. 1995) or hybridization technique (LARSEN et al. 2000) is used. Microarrays (CHIP-DNA) are recently used to type *S. aureus* strains through hybridization (LISOWSKA-ŁYSIAK et al. 2019). Another method is fingerprinting based techniques – which involves amplifying a polymorphic DNA fragment in a PCR reaction using appropriately designed primers. In the *S. aureus* typing, the presence in the genome of tandem repetitive substances (VNTR, Variable – Number Tandem Repeat) (SABAT et al. 2006, PICHETTE-JOLETTE at al. 2019) can be used. Among them *ssp, coa, spa, sdr* regions or the AFLP (Amplified Fragment Length Polymorphism) method (SAKWIŃSKA et al. 2011, VAN LEEUWEN et al. 2005) and ADSRRS fingerprinting (Amplification of DNA fragments Surrounding Rare Restriction Sites) (KRAWCZYK et al. 2007).

Recently the methods utilizing Multi-Locus Sequence Typing (MLST) (SABAT et al. 2013, JAGIELSKI at al. 2014, RABELLO et al. 2015) are gaining significance. It is low cost, easy and fast as well as a highly repeatable method. A complementary method to MLST typing is *spa typing*, which is increasingly used in typing staphylococcal strains isolated from cows with mastitis (LISOWSKA-ŁYSIAK et al. 2019). Due to the increasingly common methicillin resistance and the associated presence of the *mec* gene, it has become necessary to subtype the variable elements responsible for methicillin resistance called the chromosomal staphylococcal mec cassette (SCCmec), however, for the correct classification of the clone MLST and *spa typing* as well as SCC-mec PCR based methods should be performed. Many phenotypic and genotypic methods are used simultaneously for the proper assessment and typing of staphylococcal strains, including those isolated from cows' mastitis.

The aim of the study was to assess the incidence of bovine mastitis caused by *S. aureus* in Poland, and to evaluate strain diversity based on biotyping, according to Umeki and ADSRRS-fingerprinting.

Materials and Methods

Isolation and Identification of *S. aureus* Strains

The research material consisted of 9,617 quarters milk samples from cows with suspected mastitis among which 1,330 strains of *S. aureus* were isolated, which were subjected to further testing. Isolation and identification of strains was carried out in accordance with the recommendations of MALINOWSKI and KŁOSOWSKA (2002). The study was performed in the Bacteriology Laboratory of the Veterinary Hygiene Institute in Łomża (Poland). The material was plated on blood agar medium and Chapman medium (BIOMED), and then incubated 24 h at 37°C under aerobic conditions. Gram staining preparations were made from a single colony after incubation. After finding Gram-positive spherical bacteria, an additional catalase test was performed using 3% hydrogen peroxide (Catalase Test Difco). Additional tests were used to identify isolated Gram-positive bacteria: a coagulase test using a classic tube test using freeze-dried plasma (Biomed S.A. Kraków), detection of the clumping factor according to KĘDZIA (1997), production of hemolysins on agar medium with the addition of 5% defibrinated sheep blood. In order to identify microorganisms more accurately, API-Staph (Bio Merieux) biochemical tests were performed.

The Ability to Produce β-lactamase

The ability to produce penicillinase by the tested strains of *S. aureus* was determined using BR66A β-lactamase identification sticks (OXOID) – Identification Sticks β-Lactamase (Nitrocefin).

Lipase Production

The lipase production capacity was tested on Baird-Parker medium (BBL) with the addition of 5% egg yolk. Tested staphylococcal liquid cultures were inoculated on Baird-Parker medium. Plates were incubated for 48 hours at 37°C. Turbidity around the colony was considered positive.

Biotyping According to Umeki Method

The biotyping according to Umeki involves classifying strains into one of the four biotypes based on the disintegration of three sugars. Biotype I decomposes- mannitol, biotype II decomposes mannose, biotype III decomposes mannitol and mannose, biotype IV decomposes mannitol, mannose, and ribose (Table 1). The decomposition of sugars was made on the liquid medium according to Brailey'a and Scott'a (Kędzia 1997). The basic medium was heated to boiling, next the medium was filtered through tissue paper, brought to pH 7.7–7.8 and in the end, it was sterilized in an autoclave at 120ºC for 20 minutes. According was made 100 ml 5% sugar solution.

Table 1

Biotype *Staphylococcus aureus* strains by Umeki et al. (1992)

Sugar	Biotype			
	I	II	III	IV
Mannitol	+	-	+	+
Mannose	-	+	+	+
Ribose	-	-	-	+

Explanations: + ability to decompose the tested sugar; - no ability to decompose the tested sugar

The basic medium and the sugar solution were mixed, afterwards 10 ml Andreadea's reagent was added, which earlier was sterilized by filtration methods. The Andreadea's reagent is obtained from 0.2% aqueous solution of acid fuchsin, it was neutralized with 1 M NaOH at pH 7.2. The obtained substrate was diffused into tubes. The single colony of *S. aureus* was cultured into a medium and it was incubated at 37ºC for 24 h. The discoloration from pink to yellow indicated the ability of *S. aureus* to decompose of sugar.

Genotyping by ADSRRS – Fingerprinting

From the study population of 225 strains, 45 randomly selected *S. aureus* strains were subjected to genotypic evaluation by ADSRRS – fingerprinting. The method was described in the publication of DZIEKIEWICZ-MRUGASIEWICZ et al. (2008). The original ADSRRS – fingerprinting method was developed by MASNY and PLUCIENNICZAK (2001) and its application to bacterial DNA-fingerprinting was shown by KRAWCZYK et al. (2003, 2007)

Statistical Analysis

Statistical package SPSS 12.0 was used to perform the statistical analyses and non-parametric compatibility tests Chi2 Pearson and UNINOVA.

Results

In study 9,617 samples of quarter milk, pathogenic microorganisms were found in 6,947 samples, which constituted 72.24%. *S. aureus* was detected in 1,330 milk samples, accounting for 19.14% of all isolated microorganisms. All strains tested produced coagulase, clumping factor, degraded mannitol and caused beta hemolysis. In this study 225 *S. aureus* strains were biotyped according to Umeki, 115 (51.11%) of *S. aureus* strains were classified into biotype III and 91 strains (40.44%) were included in biotype IV, nine strains (8.45%) belonged to biotype I, while no strain was classified as biotype II. The affiliation of the tested *S. aureus* strains to a specific biotype according to Umeki is shown in Table 2.

Table 2

Affiliation of *Staphylococcus aureus* strains isolated from cow's milk samples to the biotype by UMEKI et al. (1992)

	Biotypes according to UMEKA (1992)			
	I	II	III	IV
S. aureus strains $N = 225$	19 (8.45%)	0 (0%)	115 (51.11%)	91 (40.44%)

In the next stage of the study, the strain belonging to a particular biotype and selected pathogenic features were compared. Biotype I included strains with the least pathogenicity features, in this group only 37% of the strains produced lipase, 12.5% produced β-lactamase. The biotype III strains produced 37.04% of lipase, 44% produced β-lactamase, biotype IV

staphylococci showed the most pathogenicity, 62.96% of them produced lipase, 66.67% had the ability to produce β-lactamase.

Genotyping examination by the ADSRRS method – fingerprinting showed that biotype III was the most diverse, it included seven different genotypes: A, B, C, D, E, F, H, of which 17 (38.6%) belonged to genotype D, five to A, two – C, three – E and one for B, I and F. Three different genotypes D, E and I belonged to biotype IV, and 12 strains were classified as genotype D, and one for E and I. Other genotypes A, B, C, E, F, H belonged mainly to biotype III. 17 (58.6%) strains belonging to genotype D were mainly classified to biotype IV 17 (58.6%), 12 (41.4%) to biotype III, no strain from this group was classified to biotype I (Table 3).

Table 3

Differentiation of *S. aureus* strains based on their classification to biotypes and genotypes

Genotype	Number of strains n = 45	Biotype		
		I n [%]	III n [%]	IV n [%]
A	5	0 0	5 100%	0 0
B	1	0 0	1 100%	0 0
C	2	0 0	2 100%	0 0
D	29	0 0	12 41.38%	17 58.62%
E	4	0 0	3 75%	1 25 %
F	1	0 0	1 100%	0 0
G	1	1 100%	0 0	0 0
H	1	0 0	1 100%	0 0
I	1	0 0	0 0	1 100 %
Total	45	1 2.22%	25 56.56%	19 42.22%

As in the case of Umeki biotyping, the majority of genotype D strains (41.4%) showed the ability to produce β-lactamase, among the remaining genotypes β-lactamase was produced only by 18.8% of the strains.

Discussion

In Poland and in the world, the occurrence of mastitis caused by *S. aureus* varies and depends on: the region, the breed of cows, environmental conditions and *mastitis* control programs. At the beginning of the 90s, infections of *S. aureus* in the Podlasie region constituted only 5.7% (JAKUBCZAK et al. 1998), since 1997 *S. aureus* already constituted 18.7% (JAKUBCZAK et al. 2001), in later studies in this region, the percentage of isolation of *S. aureus* averaged 19.14%, of which 28.15% of isolated strains in the last year of the study. In subsequent years, the percentage of mammary gland infections with this microorganism in the studied region remained at a similar level (19.8%) (NIEDZIELA et al. 2008). During the analyzed period, *S. aureus* was most often isolated in northern Poland, namely 34.8–50.7% of samples delivered to the laboratory in Malbork (CZUPA and CZUPA 2001). In the Bydgoszcz region, MALINOWSKI et al. (2003) classified 34.5% of the strains as *S. aureus*, while in subsequent analyzes only 8.6% (MALINOWSKI et al. 2006), while in the Lublin region 10.4% (KRUKOWSKI et al. 2000). Recent studies have shown that the percentage of isolation of *S. aureus* in the country is at a similar level, namely 13% (SMULSKI et al. 2011) and 12.1% (SZTACHAŃSKA et al. 2016) in the region of north-eastern Poland and 20.8% (BURMAŃCZUK et al. 2016) in the West Pomeranian Voivodeship. In the research of Rola et al. (2015) involving small farms, as much as 50% of milk samples were infected with *S. aureus*. It should be noted that the latest data related to research carried out at specific farms, while the previous data concerned mainly analyzes of the number (percentage) of inflammations occurring during the year generally (SMULSKI et al. 2011). The author also noted that larger farms had better health status. In the world literature, the occurrence of mammary gland infections caused by *S. aureus* is diverse. Currently, following the introduction of *mastitis* eradication plans, the percentage of isolation of *S. aureus* from the mammary gland of cows is lower.

Understanding the etiology of mammary gland inflammation in the studied region allows establishing a strategy for controlling and preventing mastitis, which is important in the case of *S. aureus*, which often causes subclinical infections. In addition, bacterial differentiation is an essential tool to control the spread, carrier of *S. aureus* strains and in epidemiological research. A properly selected typing method allows you to show the direction of infection spread and indicate sources of origin of a given strain (MIĘDZYBRODZKI et al. 2008). In the past, phenotypic methods dominated in strain typing, currently genotypic methods are commonly used to differentiate microorganisms. Phenotyping of *S. aureus*

strains and especially biotyping (HAJEK and MARSALEK 1971, DEVRIESE et al. 1984) is mainly a historical method due to high variability, low accuracy, poor repeatability of results and low discriminatory power (TENOVER et al. 1994) as well as occurrence of *S. aureus* strains with atypical biochemical features (MIĘDZYBRODZKI et al. 2008). Despite this, research continues to use these methods as a complement to genotyping (MYLLES et al. 1997) and more recently Hakimi et al. (2016). The authors compared Devriese biotyping methods of *S. aureus* strains to genotypic methods such as RAPD PCR (MYLLES et al. 1997), PCR (HAKIMI et al. 2016). Both noticed the relationship between given phenotypic traits and the ability to spread and transmit strains. In microbiological studies, the occurrence of specific phenotypic traits is often used, e.g. biofilm production capacity (FOX et al. 2005), toxin production capacity (KOT et al. 2011) or antibiotic sensitivity (JAGIELSKI et al. 2014) compared to studies with application of genetic methods. The literature often compares the occurrence of given phenotypic traits and genotypic methods.

In our own research, two little-known typing methods were used: Umeki biotyping involving the breakdown of 3 sugars and ADSRRS fingerprinting genotyping. As a result of biotyping according to Umeki, 8.45% of the tested strains were included in the biotype I, they showed the least pathogenic characteristics, no strain was classified to biotype II, 51.11% was included in III and 40.44% of the tested strains in IV. The obtained results show similarity to the research of Umeki et al. (1992), in which biotype III was also the dominant biotype, strains of this biotype were found on all farms. Rarely, strains belonging to biotype II (4.6%) were isolated, and biotype I and IV represented 16.9% of the strains. Other results were obtained by SACHANOWICZ and JAKUBCZAK (2004), who most often detected strains of biotype IV – 55.8%, III – 20.3%, II – 1.3% and I – 17%, respectively. In addition, UMEKI et al. (1993) studied the relationship between belonging to a given biotype and the occurrence of selected pathogenicity features. They noticed that the ability to produce coagulase, lipase and fibrinolysin in staphylococci increased with belonging to a higher biotype, reaching the highest values among strains belonging to biotype IV. This thesis is confirmed by our own research, which showed that as much as 62.96 % of *S. aureus* strains of this biotype produced lipase, 66.67 % had the ability to produce beta-lactamase. Among strains belonging to biotype I, only one strain (12.5%) produced β-lactamase by analogy, 37% produced lipase, slime, and biofilm also only 1 strain, which accounted for 12.5%. The results obtained coincide with those of SACHANOWICZ et al. (2003) and UMEKI et al. (1992) which confirm the thesis on the relationship between the virulence of the *S. aureus* strain and belonging to a specific biotype.

In own research, strains belonging to individual biotypes were subjected to ADSRRS fingerprinting genotyping. This technique was developed by Polish scientists MASNY and PŁÓCIENNICZAK (2001), modified by KRAWCZYK et al. (2003). The ADSRRS fingerprinting was used in human medicine to differentiate clinical strains of *E. coli, Klebsiella oxytoca, Klebsiella pneumoniae* (MASNY and PŁÓCIENNICZAK 2001, KRAWCZYK et al. 2005), *Serratia marcescens, Enterococcus faecium* (KRAWCZYK et al. 2003) as well as *S. aureus* isolated from skin furuncles (KRAWCZYK et al. 2007). The method has also been used in veterinary medicine to differentiate *Corynebacterium pseudotuberculosis* strains isolated from goats (STEFAŃSKA et al. 2007). Whereas NOWAKIEWICZ et al. (2017) used this method for typing *Enterococcus* strains isolated from pigs and in the assessment of *Staphylococci* isolated from wild animals (2016). This method was first used for typing *S. aureus* strains isolated from milk of cows with mastitis by DZIEKIEWICZ-MRUGASIEWICZ et al. (2008), where studies have shown the occurrence of nine different genotypes marked from A to G. The dominant was the D genotype, which was characterized by high pathogenicity and spreading ability, namely in the first year of the study the D genotype isolates constituted 14.29%, and in the second year of the study 73.64% of all isolates. Moreover *S. aureus* strains belonging to D genotype had higher ability to adhere to the mammary epithelial cells and slime and biofilm production. The next study compared the belonging of *S. aureus* strains of individual genotypes to biotypes according to Umeki. The results showed that all genotype D strains belonged to pathogenic biotypes III and IV, most of which belonged to biotype IV. This is confirmed by the relationship between phenotypic and genotypic traits, which affects the ability of the strains to spread.

Own and other authors' research showed the usefulness of the ADSRRS fingerprinting method in strain typing and epidemiological analyzes. NOWAKIEWICZ (2016) additionally showed a strong correlation between genotype and phenotype profile together with antibiotic resistance. In contrast, STEFAŃSKA (2007) showed that ADSRRS fingerprinting has a higher discriminatory power, better repeatability than RAPD-PCR and Box PCR methods. KRAWCZYK et al. (2007) showed that ADSRRS fingerprinting has similar discriminatory power as PFGF considered as the gold standard in genetic testing. Currently, molecular based methods such as MLST or *spa* typing predominate in molecular research, they are more accurate and have a high discriminatory power, unfortunately there are no studies comparing both techniques.

Conclusion

Mastitis caused by *S. aureus* is the most common problem in dairy farming in Poland and in other countries. Umeki biotyping and ADSRRS--fingerprinting genotyping can be useful method to typing *S. aureus* strains. In addition, the ADSRRS-fingerprinting can be used in the study of etiological factors. The advantage of both methods is the ease of implementation, low costs, and no need to have specialized equipment.

Accepted for print 30.04.2020

References

Aarestrup F.M., Wegener H.C., Jensen N.E., Jonsson O., Mylles V., Thorberg B.M., Waage S., Rosdahl V.T. 1997. *A study of phage – and ribotype patterns of Staphylococcus aureus isolated from bovine mastitis in the Nordic countries*. Acta Vet. Scand., 38: 243–245.

Aarestrup F.M., Wegener H.C., Rosdahl V.T. 1995. *Evaluation of phenotyping and genotyping methods for epidemiological typing of Staphylococcus aureus isolates from bovine mastitis in Denmark*. Vet. Microbiol., 45: 139–150.

Ambroggio M.B., Perhrig MS, Camussone C., Pujato N, Berton., Gianneechini E., Alvarez S., Marcipar I.S., Calvinho L.F., Barbagelata M.S. 2018. *Survey of potential factors involved in the low frequency of CP5 and CP8 expression in Staphylococcus aureus isolates from mastitis of dairy cattle from Argentina, Chile, Uruguay*. J. Appl. Genet., 59(3): 357–363.

Anderson K.L., Lyman R.L., Bodeis-Jones S.M., White D.G. 2006. *Genetic diversity and antimicrobial susceptibility profiles among mastitis-causing Staphylococcus aureus isolated from bovine milk samples*. Am. J. Vet. Res., 67(7): 1185–1191.

Annemüller C., Lammers C., Zschock M. 1999. *Genotyping of Staphylococcus aureus isolated from bovine mastitis*. Vet. Microbiol., 69: 217–224.

Aguilar B., Amorena B., Iturralde M. 2001. *Effect of slime on adherence of Staphylococcus aureus isolated from bovine and ovine mastitis*. Vet. Microbiol., 78: 183–19.

Bardiau M., Yamazaki K., Duprez J.N., Taminiau B., Mainil J.G., Ote I. 2013. *Genotyping and phenotyping characterization of methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk bovine mastitis*. Lett. Appl. Microbiol., 57(3): 181–186.

Bardiau M., Yamazaki K., Duprez J.N., Taminiau B., Mainil J.G., Ote J.G. 2013. *Genotyping and phenotyping characterization of methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk of bovine mastitis*. Lett. Appl. Microbiol., 57: 181–186.

Burmańczuk A., Kowalski C., Roliński Z., Krasucka D. 2016. *Activity of β-lactam antibiotics against certain microorganisms which cause mastitis in cows*., J. Vet. Res., 60: 267–271.

Castelani L., Santos A.F., dos Santos Miranda M., Zafalon L.F., Pozzi C.R., Pozzi Arzaro J.R. 2013. *Molecular typing of mastitis-causing Staphylococcus aureus isolated from heifers and cows*. Int. J. Sci., 14: 4326–4333.

Cucarella C., Solano C., Vvalle J., Amorena B., Lasa I., Penades J.R. 2001. *Bap, a Staphylococcus aureus surface protein involved in biofilm formation*. J. Bacteriol., 183: 2888–2896.

Czupa S., Czupa M. 2001. *Diagnostyka laboratoryjna mastitis u krów*. Życie Wet., 76: 471–473.

Devriese S., Zadik P.M. 1984. *A simplified system for biotyping Staphylococcus aureus strains isolated from animal species*. J. Appl. Bacteriol., 56: 257–220.

Dziekiewicz-Mrugasiewicz M., Rzewuska M., Stefańska I., Jakubczak A. 2008. *Wybrane cechy chorobotwórczości oraz zróżnicowanie genomowe szczepów Staphylococcus aureus wyizolowanych z mleka krów z zapaleniem gruczołu mlekowego w regionie północno-wschodniej Polski*. Med. Weter., 64(8): 1012–1015.

FIZGERALD J.R., MEANER W.J., HARTIGAN P.J, SMYTH C.J., KAPUR V. 1997. *Fine-structure molecular epidemiological analysis of Staphylococcus aureus recovered from cows*. Epidemiol. Infect., 119(2): 261–269.

FOX L.K., GERSHMAN M., HANCOCK D.D., HUTON C.T. 1991. *Fomites and reservoirs of Staphylococcus aureus causing intramammary infections as determined by phage typing: the effect of milking time procedure hygiene practices*. Cornell Vet., 81: 183–193.

FOX L.K., ZADOKS R.N., GASKINS C.T. 2005. *Biofilm production by Staphylococcus aureus is associated with intramammary infection*. Vet. Microbiol., 107: 295–299.

FROST A.J. 1975. *Selective adhesion of microorganisms to the ductular epithelium of bovine mammary gland*. Infect. Immun., 12: 1154.

GUIDRY A., FATTOM A., PATEL A., O'BRIEN C., SHEPHERD S., LUHUIS J. 1998. *Serotyping scheme for Staphylococcus aureus isolated from cows with mastitis*. Am. J. Vet. Res., 59: 1537–1539.

HAJEK V., MARSALEK E. 1971. *The differentiation of pathogenic staphylococci and a suggestion for their taxonomic classification*. Zbl. Bakt. Hyg., 217: 176–182.

HAKIMI ALNI R., MAHAMMADZADEH A., MAHMOODI P. 2016. *Biotyping of Staphylococcus aureus strains isolated from humans and bovine raw milk samples in Hamedan province*. Int. J. Health Life Sci., 2(2): e74158.

HATA E. 2016. *Bovine mastitis outbreak in Japan caused by methicillin-resistant Staphylococcus aureus New York /Japan clone*. J. Vet. Diagn. Invest., 28: 291–298.

HAYAKAWA Y., HASHIMOTO N., IMAIZUMI K., KAIDOH T., TAKEUCHI S. 2001. *Genetic analysis of exfoliative toxin A-producing Staphylococcus aureus isolated from mastitis cow's milk*. Vet. Microbiol., 78: 39–48.

JAGIELSKI T., PUACZ E., LISOWSKI P., SIEDLECKI W., DUDZIAK J., MIĘDZYBRODZKI J., KRUKOWSKI H. 2014. *Short communication: Antimicrobial susceptibility profiling and genotyping of Staphylococcus aureus isolates from bovine mastitis in Poland*. J. Dairy Sci., 97: 6122–6128.

KARAKULSKA J., POBUCEWICZ A., NAWROTEK P., MUSZYŃSKA M., FUROWICZ A.J., CZERNOMYSY-FUROWICZ D. 2011. *Molecular typing of Staphylococcus aureus based on PCR-RFLP of coa gene and RAPD analysis*. Pol. J. Vet. Sci., 14(2): 285–286.

KRAWCZYK B., NAUMIUK Ł., LEWANDOWSKI K., BARANIAK A., GNIADOWSKI M., SAMET A., KUR J. 2003. *Evaluation and comparison of random amplification of polymorphic DNA, pulsed-field gel electrophoresis and ADSRRS-fingerprinting for typing Serratia marcescens outbreaks*. FEMS., 38: 241–248.

KRAWCZYK B., LEWANDOWSKI K., BRONK M., SAMET A., MYJAK P., KUR J. 2002. *Evaluation of novel method based on amplification of DNA fragments surrounding rare restriction sites (ADSRRS fingerprinting) for typing strains of vancomycin – resistant Enterococcus faecium*. J. Microbiol. Methods., 52: 341–351.

KRAWCZYK B., SAMET A., CZARNIAK E., SZCZAPA J., KUR J. 2005. *Extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a neonatal unit: control of an outbreak using a new ADSRRS technique*. Pol. J. Microbiol., 54(2): 105–110.

KOT B., PIECHOTA M., ZDUNEK E., WOLSKA K.M., BINEK T., OLSZEWSKA J., KŁOPOTOWSKA E., ANTOSIEWICZ M. 2012. *Phenotypic and genotypic antimicrobial resistance of staphylococci from bovine milk*. Pol. J. Vet. Sci., 15(4): 677–683.

KOT B., PIECHOTA M., ANTOS-BIELSKA M., WOLSKA K.M., FRANKOWSKA A., ZDUNEK E., BINEK T., GULIŃSKI P., TRAFNY EA. 2012. *Antimicrobial resistance and genotype of staphylococcus from bovine and the cowshed environment*. Pol. J. Vet. Sci., 15(4): 741–749.

KUŹMA K., MALINOWSKI E., LASSA H., KŁOSSOWSKA A. 2005. *Analysis of protein gene polymorphism in Staphylococcus aureus isolated from bovine mastitis*. Bull. Vet. Inst. Pulawy, 49: 41–44.

LANGE C., CARDOSO M., SENCZEK D., SCHWARZ S. 1999. *Molecular subtyping of Staphylococcus aureus isolates from cases of bovine mastitis in Brazil*. Vet. Microbiol., 67: 127–141.

LARSEN H., HUDA A., ERIKSEN N., JENSEN N. 2000. *Differences between Danish bovine and human Staphylococcus aureus isolates in possession of superantigens*. Vet. Microbiol., 76: 153–162.

LEEUWEN W.B., MELLES D.C., ALAIDAN A., AL-AHDAL M., BOELENS H.A., SNIJDERS S.V., WERTHEIM H., VON DUIJKEREN E., PEETERS J.K., VAN DER SPEK P.J., GORKIN R., SIMON G.,

Verbrugh H.A., van Belkum A. 2005. *Host- and tissue specific pathogenic traits of Staphylococcus aureus.* J. Bacteriol., 187: 4584–4591.

Leitner G., Krifucks O., Glickman A., Younis A., Saran A. 2003. *Staphylococcus aureus strains isolated from bovine mastitis: virulence, antibody, production and protection from challenge in a mouse model.* FEMS Immunol. Med. Microbiol., 35: 99–106.

Lisowska-Łysiak K., Dudko P., Kosecka-Strojek M., Walczak J., Wójcik P., Międzybrodzki J. 2018. *Characteristics of advanced method used for typing bacterial isolates from mastitis with particular reference to Staphylococci.* Pol. J. Vet. Sci., 21(1): 229–239.

Lisowska-Łysiak K., Kosecka-Strojek M., Białecka J., Kasprowicz A., Garbacz K., Piechowicz L., Kmet V., Savini V., Międzybrodzki J. 2019. *New insight into genotypic and phenotypic relatedness of Staphylococcus aureus strains from human infections or animal reservoirs.* Pol. J. Microbiol., 68(1): 93–102.

Łopaciuk U., Dzierżanowska D. 2001. *Gronkowce metycylinooporne: mechanizmy oporności, czynniki zjadliwości oraz metody genotypowania.* Post. Mikrobiol., 41(4): 401–418.

Mackie D.P., Pollock D.A., Rodgers S.P., Logan E.F. 1987. *Phage typing of Staphylococcus aureus associated with subclinical bovine mastitis.* J. Dairy. Res., 54: 1–5.

Malinowski E., Kłossowska A. 2002. *Diagnostyka zakażeń i zapaleń wymienia.* PIW, Puławy.

Matthews K.R., Jayarao B.M., Oliver S.P. 1993. *Plasmid pattern analysis of Staphylococcus aureus isolated from bovine mammary secretions.* J. Dairy Sci., 75: 3318–3323.

Matthews K.R., Jayarao B.M., Oliver S.P. 1992. *Restriction endonuclease fingerprinting of genomic DNA of Staphylococcus species of bovine origin.* Epidemiol. Infect., 109: 59–68.

Melchior M.B., Vaaarkamp H., Fink-Gremmels J. 2006. *Biofilm: A role in recurrent mastitis infections?* Vet. J., 171: 398–407.

Międzybrodzki J., Małachowa N., Markiewski T., Białecka A., Kasprowicz A. 2008. *Różnicowanie izolatów Staphylococcus aureus w oparciu o cechy fenotypowe*. Postępy Hig. Med. Dośw., 62: 322–327.

Myllys V., Ridell J., Bjorkroth J., Biese I., Pyorala S. 1997. *Persistence in bovine mastitis of Staphylococcus aureus clones as assessed by random amplified polymorphic DNA analysis, ribotyping and biotyping.* Vet. Microbiol., 57(2–3): 245–51.

Nascimento J., dos S., Giambiagi-deMarval M., de Oliveira S.S., Caotto H., dos Santos K.R., Bastos Mdo C. 2005. *Genomic fingerprinting of bacteriocin-producer strains of Staphylococcus aureus.* Res. Microbiol., 156(8): 837–842.

Nawrotek P., Karakulska J., Czernomysy-Furowicz D., Borkowski J., Furowicz A. 2009. *Typowanie molekularne szczepów Staphylococcus aureus z mleka krów chorych na mastitis.* Med. Weter., 65(2): 127–130.

Niedziałek G., Frankowska A., Karwowski Ł., Kostiuk S. *Problemy mastitis u krów z regionu północno-wschodniej Polski.* 2012. Nukowyj Wisnk., 14(52): 396–401.

Nowakiewicz A., Ziółkowska G., Zięba G., Gnat S., Wojtanowicz-Markiewicz K., Trościańczyk A. 2016. *Coagulase-positive Staphylococcus isolated from wildlife: Identification, molecular characterization and evaluation of resistance profiles with focus on a methicillin-resistant strain*. Comp. Immunol. Microbiol. Infect. Dis., 44: 21–28.

Nowakiewicz A., Ziółkowska G., Trościańczyk A., Zięba G., Gnat S. 2017. *Determination of antimicrobial resistant of Enterococcus strains isolated from pigs and their genotypic characterization by method of amplification of DNA fragment surrounding rare restriction sites (ADSRRS fingerprinting).* J. Med. Microbiol., 66: 175–183.

Pichette-Jolette S., Millette E., Demontier E., Bran-Barrera D., Cyrenne M., Ster C., Haine D., Keefe G., Malouin F., Roy J.P. 2019. *Partial prediction of duration and clinical status of Staphylococcus aureus bovine intramammary infection based on phenotypic and genotyping analysis of isolates.* Vet. Microbiol., 228: 188–195.

Poutrel B., Boutonnier A., Sutra L., Fournier J.M. 1988. *Prevalence of capsular polysaccharide types 5 and 8 among Staphylococcus aureus isolated from cow, goat and ewe milk.* J. Clin. Microbiol, 26(1): 38–40.

Puacz E., Ilczyszyn W.M., Kostecka M., Buda A., Dudziak W., Polakowska K., Panz T., Białecka A., Kasprowicz A., Lisowski A., Krukowski H., Cuteri V., Międzybrodzki J. 2015. *Clustering of Staphylococcus aureus bovine mastitis strains from regions of Central-Eastern Poland based on their biochemical and genetic characteristics*. Pol. J. Vet. Sci., 18(2): 333–342.

Rabello R.F., Moreina B.M., Lopes R.M., Teixeira L.M., Riley L.W., Castro A.C. 2007. *Multilocus sequence typing of Staphylococcus aureus isolated recovered from cows with mastitis in Brazilian dairy herds*. J. Med. Microbiol., 56: 1505–1511.

Rola J., Czubkowska A., Korpysa-Dzirba W., Osek J. 2016. *Occurrence of Staphylococcus aureus on farms with small scale production of raw milk cheeses in Poland*. Toxins, 8(3): 62.

Sabat A., Malachowa N., Międzybrodzki J., Hryniewicz W. 2006. *Comparison of PCR-based method for typing Staphylococcus aureus isolated*. J. Clin. Microbiol., 44(10): 3804–3807.

Sachanowicz J., Jakubczak A., Kleczkowski M. 2003. *Różnicowanie szczepów Staphylococcus aureus metodą PCR-fingerprinting*. Med. Weter., 59: 710–712.

Sakwińska O., Morisset D., Madec J.-Y., Waldvogel A., Moreillon P., Haenni M. 2011. *Link between genotype and antimicrobial resistant in bovine mastitis-related Staphylococcus aureus strains, determined by comparing Swiss and France isolated from Rhone Valley*. App. Environ. Microbiol., 77(10): 3428–3432.

Salimena A.P., Lange C.C., Camussone C., Signorini M., Calvinho L.F., Brito C.A., Borges A.V., Guimaraes A., Ribeiro J.B., Mendonca L.C., Piccoli R.H. 2016. *Genotyping and phenotyping detection of capsular polysaccharide and biofilm formation in Staphylococcus aureus isolated from bovine milk collected from Brazilian dairy farms*. Vet. Res. Commun., 40: 97–106.

Da Silva R., da Silva N. 2005. *Coagulase gene typing of Staphylococcus aureus isolated from cows with mastitis in southeastern Brazil*. Can. J. Vet. Res., 69(4): 260–264.

Smulski S., Malinowski E., Kaczmarowski M., Lassa H. 2011. *Występowanie, postacie i czynniki etiologiczne zapaleń wymienia w Polsce w zależności od wielkości gospodarstwa*. Med. Weter., 57(3): 190–193.

Sordelli D.O., Buzzola F.R., Gomez M.I., Steele-Moore L., Berg D., Gentilini E., Catalano M., Reitz A.J., Tollersrud T., Denamiel G., Jeric P., Lee J.C. 2000. *Capsule expression by bovine isolates of Staphylococcus aureus from Argentina: genetic and epidemiologic analyses*. J. Clin. Microbiol., 38: 846–850.

Stefańska I., Rzewuska M., Nowicki M., Kaba J., Binek M. 2007. *Właściwości fizjologiczne i zmienność genomowa Corynebacterium pseudotuberculosis wyizolowanych od kóz w Polsce*. Med. Weter., 63(11): 1474–1477.

Stefańska I., Rzewuska M., Binek M. 2008. *Evaluation of three methods for DNA fingerprinting of Corynebacterium pseudotuberculosis strains isolated from goats in Poland*. Pol. J. Microbiol., 57(2): 105–112.

Sutra L., Mandolia C., Rainard P., Poutrel B. 1990. *Encapsulation of Staphylococcus aureus isolates from mastitis milk: relationship between capsular polysaccharide types 5 and 8 and colony morphology in serum-soft agar, clumping factor, teichoic acid and protein A*. J. Clin. Microbiol., 28: 447–451.

Sztachańska M., Barański W., Janowski T., Pogorzelska J., Zduńczyk S. 2016. *Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland*. Pol. J. Vet. Sci., 19(1): 119–124.

Szweda P., Schielmann M., Milewski S., Frankowska A., Jakubczak A. 2012. *Biofilm production and presence of ica and bap genes in Staphylococcus aureus strains from cows with mastitis in eastern Poland*. Pol. J. Microbiol, 61(1): 65–69.

Szymańska K., Buczek J. 1999. *Charakterystyka mechanizmów chorobotwórczości gronkowców*. Med. Weter., 55(9): 590–594.

Tollersrud T., Kenny K., Reitz J.R., 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*. J. Clin. Microbiol., 38: 2998–3003.

UMEKI F., OTSUKA G., SEKI M., YOSHIDA M., YOSAI A., TAKEISHI M. 1992. *Evaluation of acid production from carbohydrates and pathogenicity of Staphylococcus aureus in bovine quarter milk*. Milchwissenschaft, 48(1): 22–25.

UMEKI F., OTSUKA G., YOSAI A., SEKI M., YOSHIDA M., TAKEISHI M., KOZAKI K., KOZAKI R. 1993. *Evaluation of biological character and pathogenicity of Staphylococcus aureus isolated from healthy persons, patients, poisoned food and cows*. Milchwissenschaft, 48(10): 552–555.

VASUDEVAN P., NAIR M., ANNAMALAI T., VENKITANARAYANAN K.S. 2003. *Phenotypic and genotypic characterization of bovine mastitis isolates of Staphylococcus aureus for biofilm formation*. Vet. Microbiol., 92: 179–185.

VINTOV J., AARESTRUP F.M., ZINN CH.E., OLSEN J.E. 2003. *Association between phage types and antimicrobial resistance among bovine Staphylococcus aureus from 10 countries*. Vet. Microbiol., 95: 133–147.

YONIS A., LETNER G., HELLER D., SAMRA Z., GADBA R., LUBASHEVSKY G., CHAFFER M., YADLIN A., WINKLER M., SARAN A. 2000. *Phenotypic characteristic of Staphylococcus aureus isolated from bovine mastitis in Israeli dairy herd*. J. Vet. Med. B., 47: 591–597.