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DEVELOPMENT OF RESISTANCE TO ANTIBIOTICS IN BACTERIA *STAPHYLOCOCCUS SPP.* ISOLATED FROM MILK SAMPLES IN THE SHEEP BREEDINGS ON EAST OF SLOVAKIA

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ABSTRACT

During the last three years (2015 to 2017), the frequency of occurrence of bacteria *Staphylococcus spp.* were examined in total 3466 individual and 12 pool milk samples. Experiment was carried out in two herds of breed of sheep Improved Valaska in region of East Slovakia. Were isolated and taxonomically identified 15 species of the genus *Staphylococcus spp.* (n = 444). From the coagulase positive staphylococci (CPS), *S. aureus* was isolated during the reporting period, however, most often in the first year (45). The incidence of *S. intermedius* and *S. hyicus* has been irregular. From the coagulase negative staphylococci (CNS) (n = 288), were isolated *S. epidermidis* present in 37.5% (108), *S. scheiferi* 25.69% (74) and *S. chromogenes* 23.61% (68), while other species occurred only rarely. The bacteria *S. aureus* (n = 117) showed the highest resistance to novobiocine 14.5%, to erythromycin 12.8%, lincomycin 7.69% and also 7.69% to penicillin. In the framework of the CNS sensitivity we tested 108 strains of *S. epidermidis*, from which it was 11.1% resistant to novobiocine and 8.3% to erythromycin. Statistical comparison of the incidence of resistance to penicillin and novobiocine in *S. aureus* and *S. epidermidis* was performed using Chi square test. There was statistically significant dependence of tracked characters was confirmed on significance level $\alpha = 0.05$. Also, the incidence of intermediate sensitivity of bacteria in the *Staphylococcus spp.*, indicates the unfavourable development of resistance to the most commonly used antibiotics to treat the inflammation of the udder in sheep. It is therefore recommended to regularly check the resistance to antibiotics and often isolated bacteria CNS.

Keywords: sheep milk; staphylococci; antibiotics; resistance; mastitis

INTRODUCTION

The Staphylococcus spp. forms a group of micro-organisms, which globally represents a significant proportion in the aetiology of the sheep mastitis **(Contreras et al. 2007)**.

In particular, *S. aureus* as a coagulase positive species, were intensively studied for its pathogenicity in both, human and veterinary medicine. In recent decades however, highlighted the importance of coagulase negative staphylococci and (CNS), which were initially considered as a comensals and had a minority as the mammary gland pathogens. Their significance as a pathogen of ruminants, especially in subclinical forms of mastitis, were recorded by experts in many countries **(Pyörälä and Taponen, 2009)**.

Pitkälä et al. (2004) reported up to 50% market share of CNS from total isolation of bacterial species in Finland, while the main indicator of the subclinical mastitis is increasing of somatic cell count in milk of ruminants. Prevention and control mastitis caused by CNS (controling CNS mastitis) are complex, since epidemiology is often unclear even for the fact that the group consists of more than 40 species, the characteristics of the CNS are diverse, they can be more or less virulent **(Kiossis et al., 2007; Pyörälä and Taponen, 2009).**

Also, with reference to the specific conditions of ruminants, many authors in their works referred to as the most frequently isolated: S. epidermidis, *S. chromogenes*, *S. simulans, S. xylosus, S. haemolyticus, S. warneri*, *and S. sciuri* **(Fthenakis 1994; Ergün et al., 2009)**.

Although CNS do not have a comparable range of the virulence factors, such as the S. aureus, one of the important factors of virulence is the ability to create resistance to the antibiotic, while some were described as multiresistant **(Moniri et al., 2007)**.

Hidden intramammary infection half of the udder are referred to as sources of resistant staphylococci in sheep holdings, which in practice can be confirmed only by a bacteriological examination (Ergün et al., 2009; Kiossis et al., 2007).

Zigo et al. (2014) in their work report that the coagulase-negative staphylococci were identified in 102 (65.4%) from all 156 positive isolates. The CNS and *S. aureus* caused subacute (5.1%), subclinical (3.9%) and acute (2.4%) forms of mastitis. The most frequently isolated were *S. epidermidis*, followed by *S. chromogenes* and *S.*

xylosus from ewes with subacute and subclinical mastitis. From acute and chronical forms of mastitis were predominantly isolated *S. aureus* and *S. epidermidis*.

The aim of the work was to determine the occurrence and most common types of *Staphylococcus spp*. in the investigated individual pools and sheep's milk samples and comparison of the incidence of antibiotico-resistance of the most numerous tested species *S. aureus* and *S. epidermidis.*

MATERIAL AND METHODOLOGY

Characteristics of experimental breeds of sheep

It was one of the breed of sheep with 330 Improved Valaska sheep, and another farm with 250 sheep with a program of gradual crossing with the "Lacaune" breed. Tracking the aetiology in mastitis in the findings of the pool samples was carried out during the three seasons of the machine milking, in the holdings with technological standards in Gelnica district. Have been performed a total of 12 comprehensive examination repeatedly from April to September. A significant measure in the course of the experiment was to treat all cases of clinical mastitis solely on the basis of proven susceptibility to a range of selected antibiotics.

Testing and sampling herds sheep's milk

At the beginning, and at the end of each season were carried out a clinical examination of the udder is supplemented by a Californian Mastitis Test individual sheep's milk, and bacteriological examination of samples according to the principles as stated by the authors **Fthenakis (1994); Vasil (2004); Mørk et al. (2004).**

Emphasis has been placed on aseptic sampling and transport of mixed pools samples and individual sheep's milk samples intended for bacteriological examination.

Bacteriological examination

The inoculum of each sample of milk was inoculated on the plates with 5% blood agar, incubated at 37 °C, and after 24 hours of reading from. When the growth were more than 7 colonies from one type of colony were inoculated and cultured on selective nutrient soils. Identification of *Staphylococcus* spp. bacterial cultures was carried out according to the assessment growth of suspected bacteria on nutrient agars (5% of blood Agar, N° 110, Baird-Parker agar, Brilliance^{ž} UTI Clarity Agar (OXOID Ltd., Basingstoke, Hants, UK). The pigment formation, haemolysis, catalase positivity, Gram positivity, creation of free or coupled coagulase, and other characters, were determined. The identification of each species was made by STAPHYtest 24 and evaluated by TNW ProAuto 7.0 (Erba-Lachema, Brno, CZECH REPUBLIC) with a probability of correct designations of the kind above 90%. The functionality of the set was controlled using a strain of Staphylococcus aureus CCM 7113 (CCM, Masaryk University, Brno, CZECH REPUBLIC).

Testing of the sensitivity on antibiotics of the most numerous species of Staphylococcus

Bacteria isolated from various forms of mastitis (n = 432) and pools milk samples (n = 12), were tested in vitro by a disc method (**EUCAST, 2014)** by evaluation of the zones of inhibition to grow on Mueller-Hinton agar after 24 hours incubation at 37 °C.

To the test of sensitivity of staphylococci to fourteen antibiotics (ampicilin, amoxicillin, cefoperazone, cefoxitin, cloxacilin, erythromycin, lincomycin, neomycin, penicillin, novobiocine, oxacilin, methicilin, streptomycin and tetracycline) have been use test discs (OXOID Ltd., Basingstoke, Hants, UK) as shown in table 1. The choice of antibiotics reflects the range of which is contained in a number of intramammary products to treat mastitis, which are available in Slovakia. Sensitivity or resistance of the bacteria tested were interpreted according to the reference zones in accordance with the instructions of the **EUCAST (2014)**.

In the tests were used as control the tribes *S. aureus* CCM 5973 and *S. epidermidis* 4418. In view of the abundance of the species of the CPS, or CNS was only possible for species *S. aureus* and *S. epidermidis* in practical terms, to evaluate resistance as a percentage: a negligible
(< 0.1%), very low (0.1 – 1%), low (1 - 10%), moderate (10 – 20%), high (20 – 50%) or very high (50 – 70%).

Statistical analyses

Statistical analysis we performed using software Microsoft Excel 2007. Chi square test (χ^2test) we used to compare the individual proportions (Kabrt 2013). The dependence of the individual signs was tested at a significance level $\alpha = 0.05$, with critical value $\chi^2 = 5.991$.

Table 1 Used testing discs of antibiotics (OXOID Ltd. Basingstoke, Hants, UK).

Antibiotics	**D** (µg)	**Z** (mm)	**Antibiotics**	**D** (µg)	**Z** (mm)
Ampicilin	10	28-29	Methicilin	10	9-14
Amoxicillin	25	28-29	Neomycin	10	12-17
Cefoperazone	30	14-18	Novobiocine	5	17-22
Cefoxitin	30	23-29	Oxacilin	5	10-13
Cloxacilin	5	10-13	Penicillin	10 U	28-29
Erythromycin	10	13-23	Streptomycin	10	11-15
Lincomycin	15	9-15	Tetracycline	10	14-19

Note: D – dose of antibiotics in µg, content of one disc; Z - reference zones in mm.

RESULTS AND DISCUSSION

Table 2 gives an overview of the types of the bacteria *Staphylococcus spp*., which we have been isolated from sheep's milk, during the three years on holdings in Eastern Slovakia. In the reporting period, a total of 156 coagulase positive staphylococci were isolated of which 75% (117) was *S. aureus*, in 14% (22) has been isolated *S. intermedius*, and 10.9% (17) *S. hyicus*.

S. aureus was isolated during the reporting period, but most frequently at the beginning of the reference period (45). *S. intermedius* was isolated in the first two years of tracking, however, most in the second year (19). *S. hyicus* has been isolated only in the first year of follow-up (17). During the period considered from 288 coagulase negative staphylococci S. epidermidis was isolated in 37.5% (108), *S. schleiferi* 25.69% (74), *S. chromogenes* 23.61% (68), *S. cohnii, ssp. urealyticum* 3,82%, *S. xylosus* 2.43%, and other species occurred only rarely **(Table 2)**. **Table 3** provides an overview of the incidence of resistance to 14 tested antibiotics in the four species of staphylococci (n = 367), which were the most frequently isolated from sheep's milk, during the three years of follow-up.

The table 4 is showed the occurrence of resistance to 14 antibiotics in *S. aureus* (from CPS group), and *S. epidermidis* (most commonly occurring from 12 kinds of CNS), which have been isolated from sheep's milk in the course of three years. In the evaluation of the tests of sensitivity of the two most numerous species were numerically expressed as numbers of (S) -sensitive, (IM) – intermediate, (R) – resistant as well as the values of the resistance in percentage (%).

Staphylococcus aureus (tables 3 and 4) showed the highest resistance to novobiocine 14.5%, to erythromycin 12.8%, to penicillin, 7.69%, and 7.69% to lincomycin. As negative effect we can consider the incidence of intermediate susceptibility to novobiocine (15 strains), erythromycin (12), penicillin (14), oxacilin (11) cloxacilin and neomycine (9 strains). To others antibiotics, the incidence of resistant strains of *Staphylococcus aureus,* was relatively low.

Bogdanovičová et al. (2014) reported that the antimicrobial resistance profile of the tested S. aureus strains to different antibacterial agents revealed that 17.8% (n = 11) of the strains were resistant to at least one antibiotic.

In the framework of the coagulase negative staphylococci (tables 3 and 4) was on the sensitivity tested 108 strains from these *S. epidermidis* in 11.1% was resistant to novobiocine, and 8.3% to erythromycin, moreover, can be identified with intermediate sensitivity to the adverse ampicillin (13), lincomycin (11), erythromycin (10), amoxicilin, novobiocine and penicillin (9 tribes).

At work we are comparing the incidence of following characters (S, IS, R) in two groups, the most numerous of staphylococci *S. aureus* and *S. epidermidis* using statistical method Chi-squared test. On the significance level $\alpha = 0.05$ (5%), was recorded in twelve antibiotic substances test value ($G < \chi 2$), the statistically independence of tracked characters was confirmed. The antibiotic substance penicillin and novobiocine when applied, $G > \chi^2$, in the test groups of *S. aureus* and *S. epidermidis* statistically dependence of the observed characters was confirmed, which means that the occurrence of the characters was not random.

Table 2 The types of the bacteria *Staphylococcus spp*., which have been isolated from sheep's milk, during the three years on holdings in eastern Slovakia.

Bacteria ***Staphylococcus spp.***	**2015**	**2016**	**2017**	**Total**	**%**
S.aureus	45	39	33	117	26.4
S.intermedius	3	19	-	22	5.0
S.hyicus	17	-	-	17	3.8
S.capitis	2	-	-	2	0.5
S.caprae	5	-	-	5	1.1
S.carnosus	-	-	4	4	0.9
S.cohnii spp. urealyticum	1	-	10	11	2.4
S.condimenti	-	1	-	1	0.2
S.epidermidis	15	43	50	108	24.3
S.chromogenes	30	7	31	68	15.3
S.sciuri	2	-	1	3	0.7
S.schleiferi	4	37	33	74	16.6
S.simulans	3	-	-	3	0.7
S.warneri	-	-	2	2	0.5
S.xylosus	4	3	-	7	1.6
∑	**131**	**149**	**164**	**444**	**100.0**

Table 3 Total overview of the incidence of resistance to 14 tested antibiotics in the four species of staphylococci (n = 367), which were the most frequently isolated from sheep's milk, during the three years of follow-up.

CPS	R	n	Antibiotics													
			AMP	AML	CFP	FOX	OB	E	MY	MET	N	Nv	OX	P	S	TE
S.aureus (n = 117)	I.	45	4	3	2	1	1	3	4	1	6	9	4	10	1	3
	II.	39	2	1	1	1	1	8	3	1	5	5	1	5	-	-
	III.	33	1	-	-	-	1	4	2	-	2	3	-	3	-	-
	∑	117	7	4	3	2	3	15	9	2	13	17	5	9	1	3
S. epidermidis (n = 108)	I.	15	1	-	-	-	-	2	1	-	2	4	-	2	-	-
	II.	43	1	1	1	-	2	3	2	-	2	3	1	2	11	-
	III.	50	2	-	2	-	2	4	2	-	3	5	2	3	1	-
	∑	108	4	1	3	-	4	9	5	-	7	12	3	7	2	1
S.schleiferi (n = 74)	I.	4	-	-	-	-	1	1	-	-	1	2	-	-	-	-
	II.	37	1	-	-	-	-	1	2	-	1	1	-	1	1	-
	III.	33	1	-	-	-	1	3	1	-	3	5	1	2	-	-
	∑	74	2	-	-	-	2	5	3	-	5	8	1	3	1	-
S. chromogenes (n = 68)	I.	30	-	1	-	-	-	1	1	-	2	2	1	1	-	-
	II.	7	1	-	-	-	-	-	-	-	1	1	-	-	-	1
	III.	31	2	1	2	1	1	2	1	-	1	3	1	4	1	-
	∑	68	3	2	2	1	1	3	2	-	4	6	2	5	1	1
Resist. ∑ KNS (∑n)		250	9	3	5	1	7	17	10	-	16	26	3	15	4	2

Note: (AMP) Ampicilin 10 μg; (AML) Amoxicilin 25 μg; (CFP) Cefoperazone 30 μg; (FOX) Cefoxitin 30 μg; (OB) Cloxacilin 5 μg; (E) Erythromycin 10 μg; MY) Lincomycin 15 μg; (MET) Methicilin 10 μg; (N) Neomycin 10 μg; (Nv) Novobiocine 5 μg; (OX) Oxacilin 5 μg; (P) Penicillin 10 IU; (S) Streptomycin 10 μg; (TE) Tetracycline 10 μg.

Table 4 An overview of the sensitivity and the occurrence of resistance to 14 tested antibiotics in two types: *S.aureus* as representative of the CPS, and *S.epidermidis* as most frequently isolated from 12 species of CNS, isolated from sheep's milk in the years 2015 to 2017.

Antibiotics	*S. aureus* (n = 117)				*S. epidermidis* (n = 108)				Test*
	S	IS	R	%	S	IS	R	%	G
Ampicilin	104	6	7	6.0	91	13	4	3.7	3.910
Amoxicilin	107	6	4	3.4	98	9	1	0.9	2.439
Cefoperazone	110	4	3	2.6	99	6	3	2.8	0.620
Cefoxitin	112	3	2	1.7	106	2	-	-	2.008
Cloxacilin	106	9	2	1.7	97	7	4	3.7	0.957
Erythromycin	90	12	15	12.8	89	10	9	8.3	1.330
Lincomycin	101	7	9	7.0	92	11	5	4.6	2.095
Methicilin	115	-	2	1.7	105	3	-	-	5.103
Neomycin	95	9	13	11.1	96	5	7	6.5	2.592
Novobiocine	93	7	17	14.5	77	19	12	11.1	7.559[1]
Oxacilin	100	11	5	4.3	98	7	3	2.8	1.129
Penicillin	94	14	9	7.0	98	3	7	6.5	7.102[1]
Streptomycin	109	7	1	0.8	100	6	2	1.8	0.439
Tetracycline	102	9	3	2.6	103	4	1	0.9	2.769

Note: Sensitivity (S); Intermediate sensitivity (IS); Resistance (R);% - resistance from base n; * Chi-squared test (significance level $\alpha = 0.05$ (5%); critical value $\chi^2 = 5.991$; G – testing value).

The potential pathogenity of bacteria *Staphylococcus spp*. first and foremost, in the accompanying confirmation of virulence factors such as: an increased incidence of resistance to common antibiotics and disinfectants **(Moniri et al. 2007; Vautor et al., 2009)**, the production of biofilm **(Melchior et al. 2006)**, and the production of enterotoxins in isolated strains **(Scherrer et al. 2004)**.

CONCLUSION

By bacteriological examination of samples for the analysis of individual pools and sheep's milk, during the three seasons of machine milking were gradually isolated and taxonomically identified 15 types of bacteria *Staphylococcus spp*., (n = 444). Coagulase positive staphylococci (CPS) *S. aureus* was isolated during the reporting period, however, most often in the first year (45).

The incidence of *S. intermedius* was registered the most significant in the second year (19). *S. hyicus* has been isolated only in the first year of follow-up (17). From the coagulase negative staphylococci (CNS) (n = 288), *S. epidermidis* present in 37.5% (108), *S. schleiferi* 25.69% (74) and *S. chromogenes* 23.61% (68), while the *S. cohnii ssp. urealyticum* 3.82%, *S. xylosus* 2.43% and *S. caprae* 1.7%, other types of the rarely. The bacteria *S. aureus* (n = 117) showed the highest resistance to novobiocine 14.5%, to erythromycin 12.8%, to lincomycin and penicillin 7.69%.

The incidence of intermediate sensitivity was recorded against to novobiocine (15 strains), to erythromycin (12), to penicillin (14), to oxacilin (11) to cloxacilin and neomycin (9). In the framework of the sensitivity of the test strains with CNS has tested 108 strains of *S. epidermidis*, from which it was 11.1% resistant to novobiocin and 8.3% to erythromycin. In addition, unfavourable can mark a high intermediate sensitivity to ampicillin (13), lincomycin (11), erythromycin (10), amoxicilin, novobiocine and penicillin (9 tribes). In the work aimed at testing the top representatives of the genus *Staphylococcus spp*. is credited to relatively unfavourable evolution of resistance to the most commonly used antibiotics to treat the inflammation of the udder in sheep.

REFERENCES

Bogdanovičová, K., Skočková, A., Šťástková, Z., Karpíšková, R. 2014. Occurrence and antimicrobial resistance of staphylococcus aureus in bulk tank milk and milk filters. *Potravinarstvo*, vol. 8, no. 1, p. 97-101. https://doi.org/10.5219/363

Contreras, A., Sierra, D., Sánchez, A., et al. 2007. Mastitis in small ruminants. *Small Ruminant Res.*, vol. 68, p. 145-153. https://doi.org/10.1016/j.smallrumres.2006.09.011

Ergün, Y., Aslantas, Ö. M, Dogruer, G. et al. 2009. Prevalence and etiology of subclinical mastitis in Awashi dairy ewes in southern Turkey. *Turk. J. Vet. Anim. Sci*., vol. 33, p. 477-483.

European Society of Clinical Microbiology and Infectious Diseases (EUCAST). 2015. Disk Diffusion Method for Antimicrobial Susceptibility Testing. - Version 5.0 (January). Available from internet: http://www.eucast.org.

Fthenakis, G. C. 1994. Prevalence and aetiology of subclinical mastitis in ewes of southern Greece. *Small Rumin. Res.*, vol. 13, p. 293-300. https://doi.org/10.1016/0921-4488(94)90078-7

Kabrt, M. 2013. Aplicated statistics: Test chí-square independence in contingence table. [online]. http://www.milankabrt.cz/testNezavislosti/

Kiossis, E., Brozos, C. N., Petridou, E., Boscos, C. 2007. Program for the control of subclinical mastitis in dairy Chios breed ewes during lactation. *Small Ruminant Res*., vol. 73, p. 194-199. https://doi.org/10.1016/j.smallrumres.2007.01.021

Melchior, M. B., Fink-Gremmel, S. J., Gaastra, W. 2006. Comparative assessment of the antimicrobial susceptibility of *Staphylococcus aureus* isolates from bovine mastitis in biofilm versus planktonic culture. *In. J. Vet. Med. B Infect. Dis. Vet. Public Health*, vol. 7, p. 326-332. https://doi.org/10.1111/j.1439-0450.2006.00962.x PMid:16930277

Moniri, R., Dastegholi, K., Akramian, A. 2007. Increasing Resistant CNS in Bovine Clinical Mastitis. *Pakistan J. Biolog. Sci.*, vol. 15, 10, p. 2465-2469. https://doi.org/10.3923/pjbs.2007.2465.2469

Mørk, T., Waage, S., Tollersrud, T., Kvitle, B., Sviland, S. 2007, Clinical mastitis in ewes, bacteriology, epidemiology and clinical features. *Acta Vet.,* vol. 49, no. 23, p. 1-8. https://doi.org/10.1186/1751-0147-49-23

Pitkälä, A., Haveri, M., Pyörälä S., Myllys V. and Honkanen-Buzalski T. 2004. Bovine Mastitis in Finland 2001 - prevalence, distribution of bacteria, and antimicrobial resistance. *J. Dairy Sci*., vol. 87, p. 2433-2441. https://doi.org/10.3168/jds.S0022-0302(04)73366-4

Pyörälä, S. and Taponen, S. 2009. Coagulase-negative staphylococci\; emerging mastitis pathogens. *Vet.Microbiol*., vol.134, p.3-8. https://doi.org/10.1016/j.vetmic.2008.09.015

Scherrer, D., Corti, S., Muehlherr, J. E., Zweifel, C., Stephan, R. 2004. Phenotypic and genotypic characteristics of Staphylococcus aureus isolates from raw bulk-tank milk samples of goats and sheep. *Vet. Microbiol*., vol. 101, no. 2, p. 101-107. https://doi.org/10.1016/j.vetmic.2004.03.016 PMid:15172692

Vasiľ, M. 2004. *Dairy cows milk gland inflammation (Zápaly mliečnej žľazy dojníc).* Košice, Slovakia : University of veterinary medicine in Košice, Elektronic version, 132 p. ISBN 80-8077-005-0. (In Slovak).

Vautor, E., Cockfield, J., Marechal, C., Le Loir, Y., Chevalier, M. D., Robinson, A., Thiery, R., Lindsay, J. 2009. Difference in virulence between *Staphylococcus aureus* isolates causing gangrenous mastitis versus subclinical mastitis in a dairy sheep flock. *Vet. Res*., vol. 40, no. 6, p. 40-56.

Zigo, F., Vasil, M., Elecko, J., Farkasova, Z., Lapin, M. 2014. Production of enterotoxins of *Staphylococcus spp.* isolated from samples of sheep milk. *Potravinarstvo,* vol. 8, no. 6, p. 92-96. https://doi.org/10.5219/361

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