

Antibacterial effect of Czech and Mānuka honey on selected mastitis pathogens

Marcela Klimešová, Irena Němečková, Eva Vondrušková, Ludmila Nejeschlebová

Dairy Research Institute, Prague, Czech Republic

Received May 28, 2018

Accepted December 7, 2018

Abstract

The work is focused on the antibacterial effect of four types of blossom honey, one honeydew and two Mānuka honey (MAN100+ and MAN400+) on selected pathogenic microorganisms isolated from cow's milk (*Staphylococcus aureus* 51 and *S. aureus* 428), sheep's milk (*S. aureus* 627), and from the Czech Collection of Microorganisms (*Streptococcus uberis* CCM 4617, *Streptococcus agalactiae* CCM 6187, *Enterococcus faecalis* CCM 4224 and *Escherichia coli* CCM 4787). The concentrations of honey samples were 20% and 30%. The obtained results showed a 100% inhibitory effect of MAN400+ on all tested bacterial strains even at a concentration of 20% and also a comparable inhibitory effect of Mānuka honey with Czech honeydew. The results indicate that honey had an inhibitory effect against the tested bacterial species which may cause mastitis.

Blossom honey, honeydew, bacterial infection, mammary gland, inhibitory effect

Bacterial infection of the mammary gland (mastitis) is a frequent and costly disease not only in dairy cows, but in small ruminants such as sheep and goats, as well (Menzies and Ramanoon 2001; Leitner et al. 2004; Vyletěllová et al. 2011; Manga and Vyletěllová 2013; Gelasakis et al. 2015; Kvapilík et al. 2015). In general, mastitis is caused by pathogenic bacteria, especially *Staphylococcus aureus*, *Streptococcus uberis*, coagulase-negative staphylococci (CNS), *Corynebacterium bovis*, *Streptococcus agalactiae*, *Escherichia coli* and others (Hanuš et al. 1992; Hanuš et al. 2004; Vyletěllová-Klimešová et al. 2014; Bogdanovičová et al. 2016). Due to the increasing number of antibiotic-resistant strains in both human and veterinary medicine, research is focused on the use of alternative antibiotic-free treatments. Among the resistant bacteria causing serious diseases are mainly methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus* spp., multi-resistant *Mycobacterium tuberculosis* or carbapenem-resistant bacteria of the genus *Enterobacteriaceae* (Rattan et al. 1998; Lukášová and Šustáčková 2003; Vanderhaeghen et al. 2010; Gupta et al. 2011). One possibility of preventing mammalian infection is to use the antimicrobial effect of animal products containing plant substances, such as honey. Cooper et al. (1999) tested the sensitivity of 58 *S. aureus* strains isolated from infected wounds to Mānuka and pasture honey and found no significant differences among the isolates in sensitivity to honey. Ali et al. (2005) also confirmed the inhibitory effect of honey, declared as fennel, on gram-positive and gram-negative mastitis pathogens (*S. aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. agalactiae*, *Proteus* spp.). The use of honey in practice is mainly spread in New Zealand, where Mānuka honey is exclusively used for the treatment of mastitis infections (Allen and Molan 1997). However, the question remains whether any local honey could be as effective as Mānuka honey.

This work was therefore focused on verifying and comparing the antimicrobial effect of different types of honey originating from northern Moravia and New Zealand on selected bacterial pathogens that can cause mastitis.

Address for correspondence:

Marcela Klimešová
Dairy Research Institute, Ltd.
Ke Dvoru 12a, 160 00 Prague, Czech Republic

Phone: +420 608 260 395
E-mail: marcela.vyletelova@seznam.cz
<http://actavet.vfu.cz/>

Materials and Methods

The origin of bacterial strains and honey samples

The tested strains were isolated from cow's milk suspected of mastitis (*S. aureus* 51 and *S. aureus* 428) and from sheep's milk (*S. aureus* 627). Other strains originated from the Czech Collection of Microorganisms in Brno (CCM; *S. uberis* CCM 4617, *S. agalactiae* CCM 6187, *Enterococcus faecalis* CCM 4224 and *E. coli* CCM 4787).

Seven kinds of honey (five from small sellers and two commercial) were tested: blossom honey K [the main content (38%) of pollen from the *Pyrus/Prunus-T* family], BK (with 62% of pollen from the *Cruciferae/Brassicaceae* family), S (with 82% of pollen from *Cruciferae* family) and MM (with 75% content of *Cruciferae* family pollen), honeydew M (with 74% content of *Myosotis sylvatica* pollen) and commercial Mānuka honey (MAN100+ and MAN400+, Watson & Son, New Zealand). Chemical compositions and more detailed pollen spectrum of the honey samples as measured by the Intertek Food Services, Bremen, Germany, are given in Tables 1, 2, and 3.

Table 1. Chemical composition of tested honey (Intertek, Bremen).

	Unit	BK	K	S	M	MM
Chloramphenicol	µg/kg	n.d.	n.d.	n.d.	n.d.	n.d.
HMF	mg/kg	1.8	4.3	5.4	2.1	6.3
Water content	%	15.9	15.4	15.5	15.4	15.4
pH		4.1	4.1	4.0	4.7	4.4
Diastase	DZ	16.2	10.3	14.8	20.7	21.9
Free acids	mmol/kg	12.7	15.4	15.2	28.3	18.0
Fructose F	g/100 g	37.8	39.0	38.8	33.3	38.2
Glucose G	g/100 g	39.0	37.2	38.3	28.5	36.1
Saccharose	g/100 g	n.d.	0.8	n.d.	0.7	0.5
Turanose	g/100 g	1.2	1.2	1.3	2.3	1.6
Maltose	g/100 g	1.6	1.8	n.d.	2.1	2.2
F/G ratio		0.97	1.05	1.01	1.17	1.6
Invert sugar (F+G)	g/100 g	76.8	76.2	77.1	61.8	74.3
Conductivity	mS/cm	0.21	0.18	0.15	1.07	0.32

K, BK, S, MM = blossom honey; M = honeydew; HMF = hydroxymethylfurfural; n.d. = not detected

The testing procedure for the inhibitory effect of honey

Blood agar (without the addition of blood) was prepared to contain honey at the concentrations of 20% and 30%. Honey was homogenized in sterile distilled water and added to the agar base after sterilization and cooling to 45±1 °C. Afterwards, the dilution plate method was performed (ČSN EN ISO 7218, 2008). The tested bacteria were suspended in sterile distilled water and the relevant dilution at a volume of 0.1 ml was inoculated onto the surface of agar with honey and incubated at 37 ± 1 °C for 24–48 h. The total count of colonies was determined in cfu·ml⁻¹ and the results were expressed as a percentage of inhibited bacteria. For this purpose, blood agar without the addition of honey or sugar was used as bacterial growth control. Another control modelling the osmotic pressure of honey was blood agar containing 20% and 30% of honey sugars: fructose (38.2%), sucrose (1.3%), maltose (7.3%) and glucose (31.3%) according to Belitz and Grosch (1992).

Statistical analysis

Paired *t*-test was used to compare variables between MAN400+ as a reference honey and others species of honey on the mastitis set of pathogens (MS Excel – Microsoft, Redmond, Washington, USA). *P* values < 0.05 were considered significant.

Results

The results regarding the inhibitory effect of honey samples are summarized in Table 4. For the 20% concentration of honey, the most significant inhibition (100%) of all the tested strains was found for honey MAN400+. Besides that, honey samples M, MM and MAN100+ significantly (60–100%) inhibited *S. aureus* and *Streptococcus* spp. strains while honey K was effective (89–94%) only against *S. uberis* and *S. agalactiae*.

Table 2. Pollen spectrum in the tested honey (Intertek, Bremen).

Blossom honey K	%
<i>Pyrus/Prunus-T, Rosaceae, Fruit-T</i>	38
<i>Cruciferae, Cruciferae, Crucifers (Brassicaceae)</i>	20
<i>Salix, Saliceae, Willow</i>	8
<i>Phacelia-T, Hydrophyllaceae, Bluebell</i>	4
Others	<3
Blossom honey BK	%
<i>Cruciferae, Cruciferae, Crucifers</i>	62
<i>Pyrus/Prunus-T, Rosaceae, Fruit-T</i>	20
<i>Salix, Saliceae, Willow</i>	5
Others	<3
Blossom honey S	%
<i>Cruciferae, Cruciferae, Crucifers</i>	82
<i>Pyrus/Prunus-T, Rosaceae, Fruit-T</i>	10
Others	<3
Blossom honey MM	%
<i>Cruciferae, Cruciferae, Crucifers</i>	75
<i>Pyrus/Prunus-T, Rosaceae, Fruit-T</i>	11
<i>Acer, Aceraceae, Maple</i>	7
Others	<3
Honeydew M	%
<i>Myosotis-T, Boraginaceae, Forget-me-not</i>	74
<i>Salix, Saliceae, Willow</i>	8
<i>Trifolium-T, Leguminosae, Clover-T</i>	5
<i>Umbelliferae, Umbellifers</i>	4
Others	<3

Table 3. Mānuka honey - chemical characteristics declared by the manufacturer and pollen content according to Intertek, Bremen.

	MAN400+ per 100 g	MAN100+ per 100 g
Energy	1375 kJ	1375 kJ
Proteins	0.2 g	0.2 g
Fat in total	0.9 g	0.9 g
Saturated fatty acids	0.6 g	0.6 g
Carbohydrates in total	78.5 g	78.5g
Sugar	76.2 g	76.2 g
Salt	20 mg	20 mg
Methylglyoxal	400mg/kg	100mg/kg
Pollen	<i>Leptospermum-T,</i> <i>Myrtaceae, Tea Tree (91%)</i>	<i>Leptospermum-T,</i> <i>Myrtaceae, Tea Tree (91%)</i>

For 30% concentration of honey, only samples MAN100+ and M had comparable effects with Mānuka honey MAN400+ on all the tested strains (97–100%). Except for *S. aureus* 428 and 627, honey samples MM and BK were significantly effective (68–100%, 71–98%, respectively) as well. Nevertheless, it is important to consider the relatively high inhibitory effect of control 30% sugar solution on *S. uberis* and *S. agalactiae* (22% and 28%, respectively).

Among the tested samples, Mānuka honey MAN400+ showed the highest inhibitory effect at both concentrations of 20% and 30%. The effectivity of other samples was in the sequence: honeydew M and honey MAN100+ as comparable with a very good effect, followed by honey MM, then honey BK and K, with honey S being the weakest.

No significant difference was found between MAN400+ and M at both concentrations, between MAN400+ and MM at a 20% concentration, and MAN400+ and BK and MAN100+ at a 30% concentration (Table 5).

As a whole, the microorganism-

ms most sensitive to honey were bacteria of the genus *Streptococcus* at both concentrations of honey. Furthermore, the highest inhibitory effect at the concentration of 20% was found for *Staphylococcus* strains, followed by *E. faecalis* and *E. coli*. At the higher concentration of 30%, the order of sensitivity was as follows: *E. coli*, *E. faecalis*, and *Staphylococcus* spp.

Discussion

In general, both Mānuka honey samples showed the highest inhibitory effect at both concentrations of 20% and 30% which was most likely caused by the presence

Table 4. The results on the inhibitory effect of honey.

Species	Control (10 ³ cfu/ml)	Sugar (10 ³ cfu/ml, %)	Honey 20%					M	MAN100+	MAN400+
			K	BK	S	MM				
<i>S. aureus</i> 51	1.80	1.71 (5)	1.53 (15)	1.55 (14)	1.78 (10)	0.22 (88)	0.02 (99)	0.25 (86)	0.00 (100)	
<i>S. aureus</i> 428	1.61	1.61 (0)	1.61 (0)	1.61 (0)	1.61 (0)	0.11 (93)	0.01 (94)	0.19 (88)	0.00 (100)	
<i>S. aureus</i> 627	1.53	1.50 (2)	1.48 (3)	1.45 (5)	1.53 (0)	0.08 (95)	0.06 (96)	0.21 (86)	0.00 (100)	
<i>S. uberis</i>	1.30	1.07 (18)	0.14 (89)	0.51 (61)	0.84 (35)	0.18 (86)	0.18 (86)	0.13 (90)	0.00 (100)	
<i>S. agalactiae</i>	0.83	0.65 (22)	0.05 (94)	0.12 (86)	0.50 (40)	0.12 (86)	0.03 (96)	0.33 (60)	0.00 (100)	
<i>E. faecalis</i>	1.62	1.62 (0)	1.41 (13)	1.18 (27)	1.56 (4)	1.05 (35)	1.26 (22)	0.42 (74)	0.00 (100)	
<i>E. coli</i>	1.30	1.23 (5)	1.23 (5)	1.26 (3)	1.27 (2)	1.22 (6)	0.88 (32)	0.53 (59)	0.00 (100)	
Species	Control (10 ³ cfu/ml)	Sugar (10 ³ cfu/ml, %)	Honey 30%					M	MAN100+	MAN400+
			K	BK	S	MM				
<i>S. aureus</i> 51	1.80	1.62 (10)	1.37 (24)	0.09 (95)	1.60 (11)	0.04 (98)	0.00 (100)	0.00 (100)	0.00 (100)	
<i>S. aureus</i> 428	1.61	1.61 (0)	1.45 (10)	1.18 (27)	1.61 (0)	0.00 (100)	0.00 (100)	0.05 (97)	0.00 (100)	
<i>S. aureus</i> 627	1.53	1.50 (2)	1.38 (10)	1.35 (12)	1.48 (3)	0.12 (92)	0.02 (99)	0.03 (98)	0.00 (100)	
<i>S. uberis</i>	1.30	1.01 (22)	0.04 (97)	0.03 (98)	0.22 (83)	0.14 (89)	0.01 (99)	0.00 (100)	0.00 (100)	
<i>S. agalactiae</i>	0.83	0.60 (28)	0.03 (96)	0.03 96	0.48 (42)	0.12 (86)	0.00 (100)	0.02 (98)	0.00 (100)	
<i>E. faecalis</i>	1.30	1.07 (18)	0.84 (35)	0.08 (94)	1.07 (18)	0.22 (83)	0.01 (99)	0.00 (100)	0.00 (100)	
<i>E. coli</i>	0.44	0.39 (13)	0.06 (87)	0.13 (70)	0.28 (36)	0.14 (68)	0.01 (98)	0.00 (100)	0.00 (100)	

K, BK, S, MM= blossom honey; M = honeydew; MAN = Mānuka honey; 100+ and 400+ = honey with a minimum content of methylglyoxal 100 mg/kg and 400 mg/kg, respectively; cfu = colony forming units

of methylglyoxal (MGO). This substance was identified as the predominant antibacterial component of Mānuka honey (Chaki et al. 2010; Hayashi et al. 2014). Most publications on the antibacterial effect of honey are dedicated to gram-positive bacteria, especially *S. aureus*, and their results are comparable with our findings. Almasaudi et al. (2017) compared the effect of honey samples against *S. aureus*. Five types of honey (Mānuka honey UMF +20/MGO 829+, Mānuka honey UMF +16/MGO 572+, Active +10/MGO 263+ Mānuka honey, Sidr honey and *Nigella sativa* honey) were evaluated for their bactericidal/bacteriostatic activities against both methicillin-resistant and sensitive *S. aureus*. The inhibitory effect of honey was evident at concentrations of 20% and even 10%. Mānuka honey showed the best results. Cooper et al. (1999) tested the susceptibility of 58 *S. aureus* strains isolated from infected wounds to Mānuka and pasture honey and reported that these honey types could prevent *S. aureus* from growing even when diluted with body fluids seven to fourteen times. The authors declared, that the antibacterial effect of pasture honey consists in the release of hydrogen

Table 5. Comparison of efficacy between honey types and MAN400+.

	Honey 20%				Honey 30%			
	d (%)	SD (%)	t	P	d (%)	SD (%)	t	P
K - MAN400+	-68.7	± 41.5	4.06	< 0.01	-48.6	± 40.6	2.93	< 0.05
BK - MAN400+	-72.0	± 33.1	5.32	< 0.01	-29.7	± 36.2	2.01	ns
S - MAN400+	-87.0	± 17.1	12.44	< 0.001	-72.4	± 29.1	6.10	< 0.001
MM - MAN400+	-30.1	± 34.9	2.12	ns	-12.0	± 10.7	2.74	< 0.05
M - MAN400+	-25.0	± 33.2	1.85	ns	-0.7	± 0.8	2.31	ns
MAN100+ - MAN400+	-22.4	± 13.4	4.11	< 0.01	-1.0	± 1.3	1.90	ns

d = arithmetic mean; SD = standard deviation; t = paired t-test; ns = non-significant; P = probability value of significance

peroxide, which can be reduced *in vivo* by the catalase activity in tissues or blood. The effect of Mānuka honey originates partly from hydrogen peroxide and partly from the phytochemical component MGO, so this type of honey may be more effective *in vivo*. In their later work, Cooper et al. (2002) tested Mānuka and pasture honey against eighteen strains of methicillin-resistant *S. aureus*, seven strains of vancomycin-sensitive enterococci isolated from infected wounds and twenty strains of vancomycin-resistant enterococci isolated from hospital environmental surfaces using an agar incorporation technique to determine minimum inhibitory concentration (MIC). For all of the strains tested, the MIC values for Mānuka and pasture honey were below 10% (v/v). However, the concentration of artificial honey required to achieve equivalent inhibition *in vitro* was at least three times higher. Mousa et al. (2012) tested different concentrations of honey (undiluted honey, 10%, 30%, 50% and 70% v/v) against *S. aureus* and *Streptococcus pyogenes* by the disc diffusion method *in vitro* and concluded that these honey samples were comparable with standard antibiotics as ampicillin, penicillin G, amoxicillin, gentamicin, tobramycin, erythromycin and chloramphenicol.

In this study, comparable results with MAN 100+ were achieved by honeydew M. Its high activity can be explained by a higher content of mineral substances, which is typical for honeydews. Likewise, high inhibitory effect was found for MM honey containing the highest content of mineral substances among the tested blossom honey samples. This characteristic is manifested by higher conductivity (0.32 mS/cm). Honey B and BK showed similar effects at a 20% concentration; however, at a 30% concentration, honey BK was more effective. This difference can be explained by the pollen composition and contents of hydroxymethylfurfural and diastase. The weakest inhibitory effect was shown in honey S, although its composition was very similar to honey BK. It differed only in a higher content of pollen grains of the family *Crucifereae* and a lower content of family *Rosaceae*.

The antibacterial influence of honey samples was confirmed on both gram-positive and gram-negative bacteria in our study. Wilkinson and Cavanagh (2005) tested the antibacterial effect of thirteen honey samples (including three commercial ones) on gram-negative bacteria *E. coli* and *P. aeruginosa*, achieving results comparable with our findings on *E. coli*. The antibacterial activity of honey was tested using standard diffusion methods with honey samples at four

concentrations (10%, 5%, 2.5% w/v). All of the tested honey types had an inhibitory effect on the growth of *E. coli* and *P. aeruginosa*, one of the honey samples still acted against *E. coli* and three showed anti-*P. aeruginosa* activity at 2.5%. This study showed that several honey types, besides the commercial antibacterial honey, can inhibit *E. coli* and *P. aeruginosa* and may have a therapeutic potential in the case of Gram-negative bacteria.

The use of honey as a wound dressing is well known in both traditional and modern medicine. Many studies have reported the effectivity of honey in the removal of bacterial infections in ulcers and abscesses and indicate that it may be suitable for the intramammary treatment of mastitis. Allen and Molan (1997) tried out the sensitivity of bacteria (*Actinomyces pyogenes*, *K. pneumoniae*, *Nocardia asteroides*, *S. aureus*, *S. agalactiae*, *Streptococcus dysgalactiae*, *S. uberis*) that usually cause mastitis in dairy cows to antibacterial honey activity. The growth of all seven species tested was completely inhibited by a typical honey (with antibacterial activity attributed to its content of hydrogen peroxide) at a concentration of 10% (v/v) in agar plates. Moreover, two species were inhibited even by 5% honey.

The obtained results indicate that honey could be a suitable raw material for the production of natural sustainable products applicable as alternative therapy in the case of mastitis or during the cow's drying period, as well as for protection against the spreading of pathogenic and resistant microorganisms in dairy production. This study presents preliminary results and a more detailed study should be conducted in the near future.

Acknowledgements

This work was supported by Ministry of Agriculture of Czech Republic by the project NAZV KUS QJ1510047 and QJ1510339 and decision No. RO1418

References

- Ali M, Wahba N, Abdel-Rahman M, Abdel-Hafeez MM 2005: Antibacterial activity of honey for treatment of subclinical bovine mastitis: 1- *In vitro* study of bacterial inhibitions and chemical bioassay of some different honeys, Conference: 8th Sci. Cong., Egyptian Society For Cattle Diseases, 11-13 Dec 2005, Assiut, Egypt
- Allen KL, Molan PC 1997: The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey. New Zealand J Agric Res **40**: 537-540
- Almasaudi SB, Al-Nahari AAM, El-Ghany ESMA, Barbour E, Muhayawi SMA, Al-Jaouni S, Azhar E, Qari M, Qari YA, Harakeh S 2017: Antimicrobial effect of different types of honey on *Staphylococcus aureus*. Saudi J Biol Sci **24**: 1255-1261
- Belitz HD, Grosch W 1992: Lehrbuch der Lebensmittel - chemie. 4. Auflage. Springer-Verlag, Berlin, 966 p.
- Bogdanovičová K, Vyletěllová-Klimešová M, Babák V, Kalhotka L, Kolářková I, Karpíšková R 2016: Microbiological quality of raw milk in the Czech Republic. Czech J Food Sci **34**: 189-196
- Chaki S, Mukherjee S, Das S, Mookerjee M, Dastidar SG 2010: Evaluation of bactericidal action of methylglyoxal and its further potentiation in the presence of honey. Int J Biom Pharm Sci **4**: 66-69
- Cooper RA, Molan PC, Harding KG 1999: Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J Royal Soc Med **92**: 283-285
- Cooper RA, Molan PC, Harding KG 2002: The sensitivity to honey of gram-positive cocci of clinical significance isolated from wounds. J Appl Microbiol **93**: 857-863
- Czech technical standard ČSN EN ISO 7218 2008: Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations. Czech Standards Institute, Praha, 68 p.
- Gelasakis AI, Mavrogiani VS, Petridis IG, Vasileiou NGC, Fthenakis GC 2015: Mastitis in sheep – The last 10 years and the future of research. Vet Microbiol **181**: 136-146
- Gupta N, Limbago BM, Patel JB, Kallen AJ 2011: Carbapenem-resistant *Enterobacteriaceae*: epidemiology and prevention. Clin Inf Dis **53**: 60-67
- Hanuš O, Frelich J, Vyletěllová M, Roubal P, Vorlíček Z, Jedelská R 2004: Technologically difficult, pathogenic and food risky bacterial contamination of raw milk and other materials from dairy cow herds. Czech J Animal Sci **49**: 489-499
- Hanuš O, Žváčková I, Genčurová V, Gabriel B 1992: A relationship between milk lactose content and indicators of the mammary gland health in the first third of lactation. Vet Med **37**: 595-604
- Hayashi K, Fukushima A, Hayashi-Nishino M, Nishino K 2014: Effect of methylglyoxal on multidrug-resistant *Pseudomonas aeruginosa*. Front Microbiol **5**: 1-6

- Kvapilík J, Hanuš O, Bartoň L, Vyleťlová Klimešová M, Roubal P 2015: Mastitis of dairy cows and financial losses: an economic meta-analysis and model calculation. *Bulg J Agri Sci* **21**: 1092-1105
- Leitner G, Chaffer M, Shamay A, Shapiro F, Merin U, Ezra E, Saran A, Silanikove N 2004: Changes in milk composition as affected by subclinical mastitis in sheep. *J Dairy Sci* **87**: 46-52
- Lukášová J, Šustáčková A 2003: Enterococci and antibiotic resistance. *Acta Vet Brno* **72**: 315-323
- Manga I, Vyleťlová M 2013: A new real-time PCR assay for rapid identification of the *S. aureus*/MRSA strains. *Acta Univ Agric Silv Mendelianae Brun* **LXI**: 1785-1792
- Menzies PI, Ramanoon SZ 2001: Mastitis of sheep and goats. *Vet Clinics North Amer: Food Animal Practice* **17**: 333-358
- Mousa A, Nouredine D, Mohamed HS, Abdelmelek M, Saad A 2012: Antibacterial activity of various honey types of Algeria against *Staphylococcus aureus* and *Streptococcus pyogenes*. *Asian Pac J Trop Med* **5**: 773-776
- Rattan A, Kalia A, Ahmad N 1998: Multidrug-resistant *Mycobacterium tuberculosis*: molecular perspectives. *Emerg Inf Dis* **4**: 195-209
- Vanderhaeghen W, Cerpentier T, Adriaensen C, Hermans JK, Butaye P 2010: Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. *Vet Microbiol* **144**: 166-171
- Vyleťlová-Klimešová M, Hanuš O, Dufek A, Němečková I, Horáček J, Ponížil A, Nejeschlebová L 2014: *Staphylococcus aureus* and other pathogens in relation to breed of cattle and somatic cell count. *Bulg J Agric Sci* **20**: 1495-1500
- Vyleťlová M, Vlková H, Manga I 2011: Occurrence and characteristics of methicillin resistant *Staphylococcus aureus* and methicillin resistant coagulase-negative *Staphylococci* in raw milk manufacturing. *Czech J Food Sci* **29**: 11-16
- Wilkinson JM, Cavanagh HM 2005: Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *J Med Food* **8**: 100-103