Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at a goat breeding farm

Z. Stastkova[1], S. Karpiskova[3], R. Karpiskova[1,2]

[1]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[2]National Institute of Public Health, Prague, Brno, Czech Republic
[3]Czech Collection of Microorganisms, Institute of Experimental Biology, Masaryk University, Brno, Czech Republic

ABSTRACT: The aim of this study was to report the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) strains at a veterinary university goat breeding farm and their characteristics. A total of 278 samples collected from animals, milk, environment and farm personnel between June 2006 and March 2008 were examined. The identification of *S. aureus* isolates was performed by a species specific PCR assay. All detected isolates were tested for resistance to oxacillin and other antimicrobials by phenotypic methods and for the *mecA* gene by PCR method. Eight MRSA were detected in this study. Five of them originated from goat's milk and three were recovered from one human carrier of the farm personnel. All obtained MRSA isolates were clonally consistent and were characterized as: SCC*mec* type IV, *spa* type t064, *seb* positive and for genes encoding TSST-1, PVL and exfoliative toxins A and B negative.

Keywords: MRSA; PCR; *mecA*; Panton-Valentine leukocidin; toxic-shock syndrome toxin-1, staphylococcal enterotoxins; exfoliative toxins; SCC*mec*; PFGE; *spa* type

The emergence of pathogenic microorganisms resistant to commonly used antibiotics is a worldwide concern of the 21st century. One of the most important bacteria in this regard is *Staphylococcus aureus*, in particular its methicillin-resistant strains. The first methicillin-resistant *Staphylococcus aureus* (MRSA) strains were isolated from hospitalized patients in the UK in 1961, i.e. only two years after methicillin had started to be used for the treatment of staphylococcal infections (Barber, 1961). Since then the prevalence of MRSA has been monitored worldwide and has shown an upward trend over the last decade. Primarily, MRSA strains have been reported to be the causative agents of hospital infections in humans. In the early 1990s, MRSA strains were isolated from the general population in the USA (Naimi et al., 2001) and in 2005 from farmed animals (Voss et al., 2005). However, detection of MRSA in animals, including also the farmed ones, was reported even much earlier (Devriese et al., 1972).

MRSA are currently also studied in veterinary medicine, mainly in food animals. MRSA-infected or colonized animals can easily be involved in the spread of the pathogen not only to farm personnel but also to raw food materials intended for further processing (Lee, 2003).

MRSA detection has been reported in cattle, horses, poultry (Devriese and Hommez, 1975; Seguin et al., 1999; Lee, 2003) and pigs (Voss et al., 2005). It has also been found in pet animals such as dogs and cats (Duquette and Nuttall, 2004) as well as in some exotic animals (O'Mahony et al., 2005).

The objective of this study was to report the detection and characteristics of MRSA strains isolated at a veterinary university farm.

Supported by the Ministry of Education, Youth and Sports (Grant No. MSM 6215712402) and the Ministry of Agriculture (Grant No. QH81111).

MATERIAL AND METHODS

Herd characteristics

White shorthaired goats have been kept at the Clinic for Ruminants at University of Veterinary and Pharmaceutical Sciences in Brno since 2004. They are kept there for educational purposes and for meat production (mainly kids). The number of adult animals is about forty. They are housed in groups of 1 to 10. The herd is in good health, no severe cases of mastitis or other diseases requiring antibiotic therapy have been observed. The goats are housed in the same building as animal patients are, but separately from the latter to prevent contact with each other. Nevertheless, the same personnel look after the animals and the same farm tools are used for both groups of animals. After weaning, the goats are machine milked twice daily and later, close to the cessation of lactation, only once daily. Milk is not further processed.

Sample characteristics

Altogether 278 samples were collected from the housing and milking shed environment (swabs), animals (nasal, rectal, conjunctival and udder swabs), farm personnel (nasal, oral, abdominal skin swabs) and laboratory staff collecting swabs, examined at the beginning and at the end of the sampling period (nasal swabs). In addition, individual and bulk tank milk samples, feed samples and animal excrement samples were collected at seven time periods from June 2006 to April 2008 (Table 1). At first only bulk tank milk samples were collected (June 2006). After positive findings of MRSA, repeated sample collections were carried out as described above.

S. aureus and MRSA detection

The following procedure was used: primary enrichment in Mueller-Hinton broth (BioRad, France) with 6.5% NaCl was followed by secondary enrichment in broth with antibiotics (TSB + 3.5 mg/l of cefoxitin + 75 mg/l of aztreonam) (LabMediaServis, Czech Republic). Simultaneous inoculation onto the Baird-Parker agar (Oxoid, United Kingdom) and onto the selective chromogenic medium ORSAB (Oxoid) was performed. Suspect colonies from both types of media were then inoculated onto blood agar (Oxoid) and assessed morphologically. *S. aureus* isolates were confirmed by the multiplex PCR method for detection of the species specific fragment SA442 (Martineau et al., 1998) and *mecA* gene, which encodes resistance to methicillin (Bosgelmez-Tinaz et al., 2006). All the *mecA* positive strains were tested for resistance to methicillin and to other groups of antimicrobials (concretely to beta-lactams, cephems, tetracyclines, sulphonamides with trimethoprim, aminoglycosides, lincosamides, glycopeptides, macrolides, fluoroquinolones, phenicols and ansamycins) by the disk diffusion method (Oxoid disks) and selected groups of antimicrobials were confirmed by MIC (E-test) (Oxoid and AB Biodisk, Sweden). The CLSI criteria (2006) were used for the result assessment. In methicillin-resistant *S. aureus* isolates, multiplex PCR was carried out to detect the presence of genes encoding enterotoxins (*sea – sej*) (Monday and Bohach, 1999; Lovseth et al., 2004), toxic shock syndrome toxin (*tst*), exfoliatins A and B (*ex*A, *ex*B) (Mehrotra et al., 2000) and Panton-Valentine leukocidin (*lukF – lukS*) (Lina et al., 1999). Furthermore, multiplex PCR typing of the staphylococcal cassette chromosome *mec* (SCC*mec*) (Milheirico et al., 2007) and macrorestriction analysis using pulsed-field gel electrophoresis (Pantucek et al., 1996) were carried out. Restriction enzyme digestion was performed using *Sma*I enzyme (BioLabs, USA) in combination with NEB4 buffer (BioLabs, USA). The assessment of pulsotypes was based on the Tenover criteria (Tenover et al., 1995). Afterwards *spa* typing (Harmsen et al., 2003) was performed in all obtained MRSA isolates.

RESULTS

Detection of *Staphylococcus aureus*

In total forty-two *S. aureus* isolates were obtained. The highest detection rates were observed in milk samples (34/153) and human samples (5/35). Only three *S. aureus* isolates were recovered from 73 animal samples (nasal, rectal, conjunctival and udder swabs) while none of the samples collected from feed, faeces and the housing and milking shed environment was positive for *S. aureus*. The results are detailed in Table 1.

MRSA detection

Eight MRSA isolates were obtained within this study. Five isolates were recovered from milk samples, i.e. four from bulk tank milk samples and one from an individual milk sample, and three isolates were of human origin. Animal records revealed that the goat with the confirmed MRSA infection (SA7 – individual milk sample) had experienced a miscarriage with retained placenta in 2008. The animal was treated with intramuscular Engemycin (oxytetracycline) for seven days. The therapy was completed two months prior to the sample collection. Neither at the time of sampling nor earlier did the animal show any symptoms of the mammary gland disease. After the positive finding of MRSA in an individual milk sample, the repeated examination of the animal was carried out in order to determine possible colonization. The examination involved the collection of nasal, rectal, conjunctival and udder swabs and the examination of faeces. No further MRSA positive sample was revealed in these samples.

The detection of MRSA in milk samples was irregular (Table 1). MRSA was first recovered from bulk tank milk in June 2006 (three positive samples), then once in June 2007 from a bulk tank milk sample and once in March 2008 from an individual milk sample.

During the study 35 human samples were taken from 17 persons of the farm and laboratory personnel involved in sampling on the farm. Three MRSA isolates were recovered, all of them originated from nasal swabs collected at different time periods from one veterinarian regularly coming in contact not only with the goats but also with other animals at the Veterinary University Clinic for Ruminants. The veterinarian was sampled three times during 2007–2008 with positive results (Table 1). This carriership is supposed to be long-term MRSA colonization; the veterinarian had neither clinical symptoms of staphylococcal infection nor other health problems.

Characterization of MRSA isolates

The eight *S. aureus* isolates carrying the *mecA* gene were also tested for oxacillin resistance by the disk diffusion method and were all oxacillin-resistant. Furthermore, the isolates showed multiresistance to beta-lactams, cephems, tetracyclines, sulphonamides with trimethoprim and aminoglycosides by the disk diffusion method, selected results were confirmed by MIC (Table 2).

All MRSA isolates carried the gene encoding enterotoxin B production (*seb*). Two of these isolates, both from bulk tank milk, also carried other genes, namely *sei* and *seg*.

None of the isolates carried any of the genes encoding the production of toxic shock syndrome toxin (*tst*), Panton-Valentine leukocidin (*lukF/S*) and exfoliatins A and B (*ex*A, *ex*B). All MRSA isolates were carriers of the same SCC*mec* type IV, all of them had the identical macrorestriction profile (pulsotype A) (Figure 1) and identical *spa* type t064 (Table 1).

DISCUSSION

In the present study *S. aureus* was detected in 14.7% of raw goat's milk samples. Similar data were reported by Zouharova and Rysanek (2008) in bulk tank cow's milk samples (15.9%). MRSA was found only in goat's milk samples and the irregular detection of MRSA in bulk tank milk samples was probably due to substantial dilution of contaminated milk and thus to substantially reduced likelihood of MRSA detection. None of the MRSA isolates was recovered from animal samples (nasal, rectal, conjunctival and udder swabs), environmental swabs and feed.

Genotyping of strains obtained within this study revealed the clonal identity of human and MRSA isolates obtained from milk. By SCC*mec* typing the strains were classified as community-associated MRSA (CA-MRSA) that are characterized by the presence of smaller cassette elements of SCC*mec* types IV and V (Boyle-Vavra and Daum, 2007). Detection of SCC*mec* type IV and V in animals was also reported in other studies (Juhasz-Kaszanyitzky et al., 2007; Khanna et al., 2008).

The way of the MRSA spread has remained unclear until now. MRSA strains were discovered only in goat's milk (in both individual and bulk tank milk samples). Milking was carried out aseptically without the presence of the veterinarian with a positive finding. The veterinarian came into the only contact with an MRSA positive goat during the treatment of retained placenta and later on after weaning. He is also expected to observe hygienic rules during manipulation or any intervention in animals and so to prevent the potential spread of

Table 1. Sampling periods, sample origin, *S. aureus* and MRSA findings and MRSA characterization

Sampling period	Sample origin		Number of samples	*S. aureus*/ MRSA	MRSA isolate	OX[1]	*mecA*	*ses*	*lukF/S*	*tst*	*exA*	*exB*	SCC*mec* type	*spa* type	Pulsotype
2006 July	milk	bulk tank sample	13	3/3	S1	+	+	*b*	–	–	–	–	IV	t064	A
					S2	+	+	*b, g, i*	–	–	–	–	IV	t064	A
					S3	+	+	*b*	–	–	–	–	IV	t064	A
2007 May	goat	nasal swab	12	0											
		rectal swab	12	0											
		udder swab	5	0											
		faeces	1	0											
	milk	individual sample	5	1/0											
	human	nasal swab	13	1/1	S4	+	+	*b*	–	–	–	–	IV	t064	A
		oral swab	3	0											
		abdominal skin swab	3	0											
	feed		1	0											
	environment	housing shed swab	5	0											
2007 June	goat	nasal swab	7	0											
		rectal swab	6	0											
		udder swab	3	0											
		faeces	1	0											
	milk	bulk tank sample	1	1/1	S5	+	+	*b, g, i*	–	–	–	–	IV	t064	A
		individual sample	3	0											
	human	nasal swab	13	3/1*	S6	+	+	*b*	–	–	–	–	IV	t064	A
		oral swab	1	0											
		abdominal skin swab	1	0											
	feed		3	0											
	environment	housing shed swab	4	0											
		milking shed swab	4	0											

Table 1. continued

Sampling period	Sample origin		Number of samples	*S. aureus*/ MRSA	MRSA isolate	OX[1]	*mecA*	*ses*	*lukF/S*	*tst*	*exA*	*exB*	SCC*mec* type	*spa* type	Pulsotype
2007 August	milk	bulk tank sample	18	13/0											
		individual sample	65	7/0											
2007 September	goat	nasal swab	9	2/0											
		rectal swab	3	0											
		conjunctival swab	3	0											
		oral swab	3	0											
	milk	bulk tank sample	2	1/0											
		individual sample	6	4/0											
2008 March	milk	individual sample	39	3/1	S7	+	+	*b*	–	–	–	–	IV	t064	A
	human	nasal swab	1	1/1*	S8	+	+	*b*	–	–	–	–	IV	t064	A
2008 April	goat	nasal swab	3	1/0											
		rectal swab	3	0											
		udder swab	1	0											
		conjunctival swab	1	0											
	milk	individual sample	1	1/0											
Total			278	42/8											

[1]oxacillin resistance tested by the disk diffusion method; *repeated recovery

Tab. 2 Minimal inhibitory concentration (MIC) of MRSA isolates of different origin

	MIC (μg/ml)					
Origin of isolate	PG	AM	TR	CI	TE	CN
Human	32	32	> 32	0.5	24	32
Bulk tank milk	48	48	> 32	0.5	24	32
Individual milk	32	32	> 32	0.5	24	32

PG = benzylpenicillin, AM = ampicillin, TR = trimethoprim, CI = ciprofloxacin (AB Biodisk strips), TE = tetracycline, CN = gentamicin (Oxoid strips)

MRSA. *Spa* type t064, the occurrence of which at the goat breeding farm is described in this study, is ranged in the group of CA-MRSA and it has not been described as livestock associated strain so far. The veterinarian regularly comes into contact also with other animals, nevertheless, they were also tested repeatedly for the presence of MRSA always with negative results (the data have not been published). Decisive data that could determine the possible origin of MRSA of the *spa* type t064 are still missing at present. In the study of Shittu et al. (2009) this type of MRSA (*spa* type t064 and SCC*mec* type IV) was reported in hospitals. On the other hand, the MRSA was negative for genes encoding staphylokinase (SAK), chemotaxis inhibitory protein of *S. aureus* (CHIPS), C3 convertase inhibitor (SCIN) and staphylococcal enterotoxin A (SEA) (not in this study), which are the four human-specific innate immune modulators typical of strains of human origin (van Wamel et al., 2006).

MRSA transmission from both farmed and pet animals to humans has already been reported (Baptiste et al., 2005; O'Mahony et al., 2005; Weese et al., 2006). MRSA transmission from farmed cows to the farm personnel has also been documented (Lee, 2003; Juhasz-Kaszanyitzky et al., 2007). The present study has confirmed the human to animal (or vice versa) transmission of MRSA and is the first report of MRSA detection from goats and/or goat's milk.

MRSA colonization is posing a risk for humans because the transmission of MRSA in community has been shown to be as high as 60%. Family members who are living with MRSA carriers are exposed to MRSA transmission (Matsumoto et al., 2001). Antibiotics were shown to be effective with eradication rates only between 53% and 85% (Roccaforte et al., 1988; Walsh, et al., 1993; Asensio et al., 1996) But antibiotics are considered to be inappropriate for patients who are only colonized and not infected with MRSA and so in view of the high likelihood that the attempts to eradicate the MRSA carriership would fail in the long run, no treatment was prescribed.

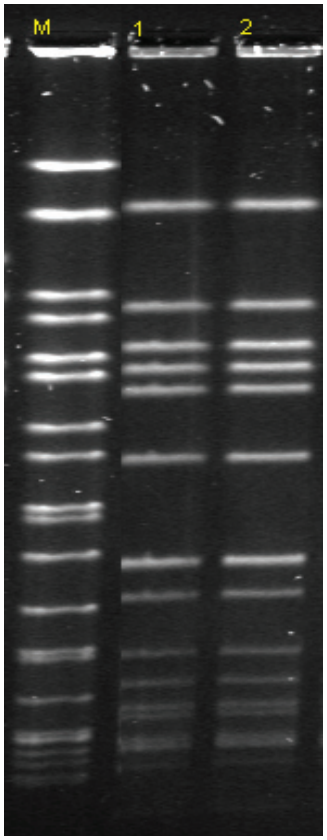

Figure 1. Macrorestriction profiles of MRSA isolates of human origin and from milk restricted with *Sma*I. Lane 1 = MRSA of human origin, lane 2 = MRSA originating from milk, *Salmonella* serotype Braenderup H 9812 restricted with *Xba*I was used as the size standard (lane M)

CONCLUSION

The presence of MRSA in basic food production poses a risk of spreading the pathogens to other animal species, humans involved in animal husbandry and food processing, foodstuffs and consequently to the general population.

Acknowledgements

The authors thank MVDr. Lubica Misurova from the Ruminant Clinic, Section of Large Animal Diseases, University of Veterinary and

Pharmaceutical Sciences in Brno, for assistance in the sampling process on the goat farm and to MUDr. Helena Zemlickova, Ph.D. from National Institute of Public Health, Prague, Czech Republic, for *spa* typing of MRSA strains.

The note of the editor

From one reviewer's point of view, it is likely a human to animal/environment transmission since based on the characteristics of the strain it is a human strain that – as stated by the authors – has not been described as livestock-associated.

REFERENCES

Asensio A., Guerrero A., Quereda C., Lizan M., Martinez-Ferrer M. (1996): Colonization and infection with methicillin-resistant *Staphylococcus aureus*. Associated factors and eradication. Infection Control and Hospital Epidemiology, 17, 20–28.

Baptiste K.E., Williams K., Willams N.J., Wattret A., Clegg P.D., Dawson S., Corkill J.E., O'Neill T., Hart C.A. (2005): Methicillin-resistant staphylococci in companion animals. Emerging Infectious Diseases, 11, 1942–1944.

Barber M. (1961): Methicillin-resistant staphylococci. Journal of Clinical Pathology, 14, 385–393.

Bosgelmez-Tinaz G., Ulusoy S., Aridoğan B., Coskun-Ari F. (2006): Evaluation of different methods to detect oxacillin resistance in *Staphylococcus aureus* and their clinical laboratory utility. European Journal of Clinical Microbiology & Infectious Diseases, 25, 410–412.

Boyle-Vavra S., Daum R. (2007): Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. Laboratory investigation, 87, 3–9.

CLSI, Clinical and Laboratory Standards Institute (2006): Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S16 [ISBN 1-56238-588-7]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087–1898 USA.

Devriese L.A., Hommez J. (1975): Epidemiology of methicillin-resistant S*taphylococcus aureus* in dairy herds. Research in Veterinary Science, 19, 23–27.

Devriese L.A., Van Damme L.R., Fameree L. (1972): Methicillin (cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. Journal of Veterinary Medicine B, Infectious Diseases and Veterinary Public Health, 19, 598–605.

Dequette R.A., Nuttall T.J. (2004): Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? The Journal of Small Animal Practice, 45, 591–597.

Harmsen D., Claus H., Witte W., Rothganger J., Claus H., Turnwals D., Vogel U. (2003): Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. Journal of Clinical Microbiology, 41, 5442–5448.

Juhasz-Kaszanyitzky E., Janosi S., Somogyi P., Dan A., van der Graaf-van Bloois L., van Duijkeren E., Wagenaar J.A. (2007): MRSA transmission between cows and humans. Emerging Infectious Diseases, 13, 630–632.

Khanna T., Friendship R., Dewey C., Weese J.S. (2008): Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Veterinary Microbiology, 128, 298–303.

Lee J.H. (2003): Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to human. Applied and Environmental Microbiology, 69, 6489–6494.

Lina G., Piemont Y., Godail-Gamot F., Bes M., Peter M.O., Gauduchon V., Vandenesch E., Etienne J. (1999): Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clinical Infectious Diseases, 29, 1128–1132.

Lovseth A., Loncarevic S., Berdal K.G. (2004): Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. Journal of Clinical Microbiology, 42, 3869–3872.

Martineau F., Picard F.J., Roy P.H., Ouellette M., Bergeron M.G. (1998): Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. Journal of Clinical Microbiology, 36, 618–623.

Matsumoto K., Hohashi N., Sugishita C. (2001): A study on the transmission of MRSA among the family members including clients of visiting nurse and related infection control. Nippon Koshu Eisei Zasshi, 48, 190–199.

Mehrotra M., Wang G., Johnson W.M. (2000): Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. Journal of Clinical Microbiology, 38, 1032–1035.

Milheirico C., Oliveira D.C., de Lencestra H. (2007): Update to the multiplex PCR strategy for assignment

of mec element types in *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy, 51, 3374–3377.

Monday S.R., Bohach G.A. (1999): Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. Journal of Clinical Microbiology, 37, 3411–3414.

Naimi T.S., LeDell K.H., Boxrud D.J., Groom A.V., Steward C.D., Johnson S.K., Besser J.M., O'Boyle C., Danila R.N., Cheek J.E., Osterholm M.T., Moore K.A., Smith K.E. (2001): Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. Clinical Infectious Diseases, 33, 990–996.

O'Mahony R., Abbott Y., Leonard F.C., Markey B.K., Quinn P.J., Pollock P.J., Fanning S., Rossney A. S. (2005): Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Veterinary Microbiology, 109, 285–296.

Pantucek R., Gotz F., Doskar J., Rosypal S. (1996): Genomic variability of *Staphylococcus aureus* and the other coagulase positive *Staphylococcus* species estimated by macrorestriction analysis using pulsed-field gel electrophoresis. International Journal of Systematic Bacteriology, 46, 216–222.

Roccaforte J.S., Bittner M.J., Stumpf C.A., Preheim L.C. (1988): Attempts to eradicate methicillin-resistant *Staphylococcus aureus* colonization with the use of trimethoprim-sulfamethoxazole, rifampin, and bacitracin. American Journal of Infection Control, 16, 141–146.

Seguin J.C., Walker R.D., Caron J.P., Kloos W.E., George C.G., Hollis R.J., Jones R.N., Pfaller M.A. (1999): Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. Journal of Clinical Microbiology, 37, 1459–1463.

Shittu A., Nubel U., Udo E., Lin J., Gaogakwe S., (2009): Characterization of meticillin-resistant *Staphylococcus aureus* isolates from hospitals in KwaZulu-Natal province, Republic of South Africa. Journal of Medical Microbiology, 58, 1219–1226.

Tenover F.C., Arbeit R.D., Goering R.V., Mickelsen P.A., Murray B.E., Persing D.H., Swaminathan B. (1995): Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of Clinical Microbiology, 33, 2233–2239.

van Wamel W.J.B., Rooijakkers S.H., Ruyken M., van Kessel K.P., van Strijp J.A. (2006): The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. Journal of Bacteriology, 188, 1310–1315.

Voss A., Loeffen F., Bakker J., Klaasen C., Wulf M. (2005): Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerging Infectious Diseases, 11, 1965–1966.

Walsh T.J., Standiford H.C., Reboli A.C., John J.F., Mulligan M.E., Ribner B.S., Montgomerie J.Z., Goetz M.B., Mayhall C.G., Rimland D., Stevens D.A., Hansen S.L., Gerard G.C., Ragual R.J. (1993): Randomized double-blind trial of rifampin with either novobiocin or trimethoprim-sulphamethoxazole against methicillin-resistant *Staphylococcus aureus* colonization: prevention of antimicrobial resistance and effect of host factors on outcome. Antimicrobial Agents and Chemotherapy, 37, 1334–1342.

Weese J.S., Dick H., Willey B.M., McGeer A., Kreiswirth B.N., Innis B., Low D.E. (2006): Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Veterinary Microbiology, 115, 148–155.

Zouharova M., Rysanek D. (2008): Multiplex PCR and RPLA Identification of *Staphylococcus aureus* enterotoxigenic strains from bulk tank milk. Zoonoses and Public Health, 55, 313–319.

Received: 2009–06–11
Accepted after corrections: 2009–09–07

Corresponding Author:

MVDr. Zora Stastkova, University of Veterinary and Pharmaceutical Sciences, Palackeho 1–3, 612 42 Brno, Czech Republic
Tel. +420 604 147 267, E-mail: stastkovaz@vfu.cz