

## Findings of Methicillin-Resistant Strains of *Staphylococcus aureus* in Livestock

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**Abstract:** The aim of our study was to determine the occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) at dairy farms in the Czech Republic. Altogether 1061 samples from 95 farms were examined. The samples analysed were milk (individual and bulk tank milk samples), animal swabs and swabs from the farm environment. In total, 299 *S. aureus* isolates were obtained, of which 23 were MRSA. These MRSA isolates originated from three farms (13 isolates from farm A and 5 isolates from each of farms B and C). All MRSA isolates carried the *mecA* gene while none of them carried the genes for PVL, TSST-1 and exfoliatins. Only the isolates from goat farm C were positive for the genes encoding enterotoxins. By SCCmec typing, the strains were classified as community-associated MRSA carrying SCCmec IV or V. This study revealed that animals can be an important source of methicillin resistant staphylococci and represent a potential hazard of further spread.

**Keywords:** MRSA; *mecA*; Panton-Valentine leukocidin; toxic-shock syndrome toxin-1; staphylococcal enterotoxins; exfoliative toxins; SCCmec; resistance

The incidence of pathogenic microorganisms that developed resistance to commonly used antibiotics has become a 21<sup>st</sup> century global issue. In this regard, the microorganisms of key importance are, above all, those of the *Staphylococcus aureus* species, especially methicillin resistant strains – MRSA. These strains were isolated for the first time in 1961 from hospital patients in the United Kingdom (BARBER 1961). Since then, global monitoring of MRSA has been ongoing and in the last decade, an increasing trend in their incidence has been observed.

Methicillin resistance in *S. aureus* is conferred by the *mecA* gene, which is itself carried in a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCCmec) (Ito *et al.* 1999). This gene is horizontally transferable and enables the

spread of methicillin resistance among staphylococci (KATAYAMA *et al.* 2000). At present, seven main types of SCCmec are recognised (DEURENBERG & STOBBERINGH 2008).

MRSA is mostly detected from humans with nosocomial infections (CARBON 2000). MRSA strains can be persistently or intermittently carried by healthy humans, and colonisation is the major risk factor for infection. Based on epidemiological and genetic characteristics, MRSA strains are divided into “hospital-acquired” (HA) and “community-associated” (CA). These groups differ from each other in pathogenicity and antimicrobial resistance. CA-MRSA are characterised by the presence of smaller cassette elements of SCCmec types IV or V and have been more often reported as producers of Panton-Valentine leukocidin (PVL)

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(NAIMI *et al.* 2003; BOYLE-VAVRA & DAUM 2007; HOSOSAKA *et al.* 2007).

Recently, MRSA strains have been monitored also in the area of veterinary medicine, especially in food-producing animals. As a result of increasing occurrence of MRSA in animals, a third group has arisen referred to as livestock-associated MRSA (LA-MRSA). Strains belonging to this group most often originate from pig farms and are multilocus sequence type 398 (ST398) (LEONARD & MARKEY 2008). MRSA in animals then poses a risk of spreading not only to other animals at farm but also to humans involved in animal care and food processing and to the general population. Infected or colonised animals can easily spread these strains not only to other animals but also to tending staff and raw materials intended for further processing (LEE 2003).

The aims of this study were to determine the occurrence of MRSA at livestock (cow, goat and sheep) farms and to characterise the obtained isolates.

## MATERIAL AND METHODS

**Samples.** Samples were collected in 2006–2009 from 95 farms in the Czech Republic: 89 cow farms, 2 goat farms and 4 sheep farms. In total, 1061 samples were collected, i.e. 469 individual milk samples, 478 bulk tank milk samples, 16 swabs from the environment and 98 animal swabs from the nasal cavity (61), rectum (24), conjunctiva (4), and udder (9).

**MRSA detection.** The following procedure was used for the detection of MRSA: primary enrichment was performed in Mueller-Hinton broth (MH, BioRad, USA) with 6.5% NaCl and secondary enrichment in broth with antibiotics (TSB + 3.5 mg/l of cefoxitin + 75 mg/l of aztreonam) (LabMedia-Servis, Czech Republic). Parallel inoculation onto the Baird-Parker agar (Oxoid, UK) and selective chromogenic medium MRSAselect (BioRad, USA) followed. Suspect colonies from both media types were then inoculated onto blood agar and assessed morphologically.

**Confirmation and characterisation of MRSA isolates.** The isolates were confirmed by the multiplex PCR method for the detection of the fragment SA 442 specific for the *S. aureus* species and the *mecA* gene which encodes the resistance to methicillin (MARTINEAU *et al.* 1998; BOŞGELMEZ-TMAZ *et al.* 2006). In methicillin-resistant *S. aureus* iso-

lates, subsequent PCRs were carried out to detect the presence of the genes encoding enterotoxins (*sea – sej*) (MONDAY & BOHACH 1999; LØVSETH *et al.* 2004), toxic shock syndrome toxin (*tst*), exfoliatins A and B (*etA*, *etB*) (MEHROTRA *et al.* 2000) and Panton-Valentine leukocidin (*pvl*) (LINA *et al.* 1999). Furthermore, multiplex PCR typing of the staphylococcal cassette chromosome *mec* (SCC*mec*) was performed (MILHEIRICO *et al.* 2007).

**Antibiotic resistance.** In the colonies that were determined as MRSA, resistance to antimicrobial agents was monitored using the disk diffusion method (disks and MH agar by Oxoid, UK). The antibiotics tested were as follows: OX – oxacillin (1 µg disk), AMC – amoxicillin/clavulanic acid (20/10 µg), FOX – cefoxitin (30 µg), CTX – cefotaxime (30 µg), TE – tetracycline (30 µg), E – erythromycin (15 µg), C – chloramphenicol (30 µg), SXT – co-trimoxazole (25 µg), DA – clindamycin (2 µg), CN – gentamicin (10 µg), CIP – ciprofloxacin (15 µg), VA – vancomycin (30 µg), TEC – teicoplanin (30 µg), (30 µg) and RD – rifampin (5 µg). Results were assessed according to CLSI (2006).

## RESULTS

In total, 299 isolates of *S. aureus* were obtained, of which 23 were identified phenotypically and genotypically as resistant to methicillin. Repeated detection of MRSA was recorded in 3 (3.2%) of 95 tested farms.

Genotypic characteristics of the obtained MRSA isolates are given in Table 1. The *mecA* gene was confirmed in all of these MRSA isolates. The genes encoding staphylococcal enterotoxins were only detected in MRSA isolates from goat farm C. The presence of any of the analysed virulence genes was not confirmed. SCC*mec* typing revealed type IV or V SCC*mec* cassettes in all MRSA isolates. The results of antimicrobial resistance testing by the disk diffusion method are also presented in Table 1. All the obtained MRSA isolates showed resistance to oxacillin, amoxicillin/clavulanic acid, cefoxitin, cefotaxime and tetracycline. Resistance to the other tested antimicrobials varied even between isolates originating from the same farm.

At farm A, eight bulk tank milk samples were collected at eight time periods between October 2008 and March 2009 and 13 MRSA isolates were identified, with five samples yielding 2 isolates each.

Table 1. Genotypic and phenotypic characterisation of MRSA isolates

MRSA	Farm	Animal	Source	Sampling date	<i>mecA</i> <sup>3</sup>	<i>ses</i> <sup>4</sup>	<i>tsf</i> <sup>5</sup>	<i>pvl</i> <sup>6</sup>	<i>etA+B</i> <sup>7</sup>	SCC <i>mec</i> <sup>8</sup>	Resistance
SA1	A	Cows	BT milk <sup>1</sup>	2008–10–16	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE
SA2	A	Cows	BT milk	2009–02–02	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN
SA3	A	Cows	BT milk	2009–02–02	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE
SA4	A	Cows	BT milk	2009–02–06	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN,
SA5	A	Cows	BT milk	2009–02–06	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, E, DA
SA6	A	Cows	BT milk	2009–02–13	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN
SA7	A	Cows	BT milk	2009–02–13	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, SXT
SA8	A	Cows	BT milk	2009–02–14	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, SXT
SA9	A	Cows	BT milk	2009–02–14	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN
SA10	A	Cows	BT milk	2009–02–24	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, SXT
SA11	A	Cows	BT milk	2009–03–02	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE
SA12	A	Cows	BT milk	2009–03–02	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, SXT
SA13	A	Cows	BT milk	2009–03–06	+	–	–	–	–	4	OX, AMC, FOX, CTX, TE, CN
SA14	B	Cows	BT milk	2008–11–20	+	–	–	–	–	5	OX, AMC, FOX, CTX, TE
SA15	B	Cows	BT milk	2009–01–22	+	–	–	–	–	5	OX, AMC, FOX, CTX, TE
SA16	B	Cows	BT milk	2009–02–04	+	–	–	–	–	5	OX, AMC, FOX, CTX, TE
SA17	B	Cows	BT milk	2009–02–20	+	–	–	–	–	5	OX, AMC, FOX, CTX, TE
SA18	B	Cows	BT milk	2009–02–13	+	–	–	–	–	5	OX, AMC, FOX, CTX, TE, E
SA19	C	Goats	BT milk	2006–07–20	+	<i>seb, sei, seg</i>	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, SXT, RD
SA20	C	Goats	BT milk	2006–07–21	+	<i>seb</i>	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, E, SXT, RD
SA21	C	Goats	BT milk	2006–07–23	+	<i>seb</i>	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, E, SXT, RD
SA22	C	Goats	BT milk	2007–07–12	+	<i>seb, sei, seg</i>	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, E, SXT, RD
SA23	C	Goats	I milk <sup>2</sup>	2008–03–27	+	<i>seb</i>	–	–	–	4	OX, AMC, FOX, CTX, TE, CN, SXT, RD

<sup>1</sup>bulk tank milk sample; <sup>2</sup>individual milk sample; <sup>3</sup>presence of gene *mecA*; <sup>4</sup>presence and types of genes encoding staphylococcal enterotoxins; <sup>5</sup>presence of gene encoding TSST-1;<sup>6</sup>presence of gene encoding PVL; <sup>7</sup>presence of genes encoding exfoliatin A and B; <sup>8</sup>type of staphylococcal cassette chromosome *mec*

OX – oxacillin (1 µg disk); AMC – amoxicillin/clavulanic acid (20/10 µg); FOX – cefoxitin (30 µg); CTX – cefotaxime (30 µg); TE – tetracycline (30 µg); E – erythromycin (15 µg); SXT – co-trimoxazole (25 µg); DA – clindamycin (2 µg); CN – gentamicin (10 µg); RD – rifampin (5 µg)

These isolates had identical genotypic characteristics, but differed in resistance to erythromycin, co-trimoxazole, clindamycin and gentamicin.

At farm B, five bulk tank milk samples were collected at five time periods between November 2008 and March 2009. All five samples were MRSA positive. Two types of isolates were obtained, differing from each other in resistance to erythromycin but having identical genotypic characteristics.

At farm C, a total of 229 samples were collected: 24 bulk tank milk samples, 119 individual milk samples, 16 swabs from the environment, 33 nasal swabs, 24 anal swabs, 9 udder swabs and 4 conjunctival swabs. Altogether 37 *S. aureus* were isolated, of these five (13.5%) were MRSA that originated from four bulk tank milk samples and one individual milk sample. The sampling was carried out at seventeen time periods from July 2006 to April 2008. The obtained isolates differed in the spectrum of the genes encoding staphylococcal enterotoxins and phenotypically in resistance to erythromycin (Table 1).

## DISCUSSION

No summary data is currently available on the incidence of MRSA or other methicillin resistant staphylococci in animals (including food production ones) or in food raw stuffs and food stuffs in the Czech Republic. Some information from abroad indicates, however, that both animal and food can take part in the spread of these dangerous bacteria. For instance, LEE (2003) has reported the detection of MRSA in cattle, pigs and chickens in Asian countries and found a relationship between animal MRSA and human infections. VOSS *et al.* (2005) have shown MRSA-colonised pigs to be the source of infection for farmers and their families. The role that animals have as a potential reservoir of MRSA that causes infection in human population is the subject matter of a series of recent research projects. In the Czech Republic, the incidence of MRSA in animals was first reported by BARDOŇ *et al.* (2006) in piglets.

In this study, none of the MRSA isolates was recovered either from animal samples (nasal, rectal, conjunctival and udder swabs) or environmental swabs. All obtained MRSA originated from milk, mostly bulk tank milk samples. In other studies carried out in cows, MRSA were most frequently isolated from milk of animals showing signs of

subclinical mastitis (LEE 2003). JUHÁSZ-KASZANYITZKY *et al.* (2007) revealed the occurrence of MRSA in cow's milk in Hungary and documented the MRSA transmission from farmed cows to farm personnel. While MRSA have been detected from both animals and the environment on pig farms, it is milk which is most often contaminated on dairy farms. As MRSA could be eliminated intermittently, the bulk tank milk sampling appears to be most suitable for MRSA screening.

None of the obtained MRSA isolates tested positive for the genes encoding TSST-1, PVL or exfoliatins. The genes encoding enterotoxins were only detected in the isolates from goat farm C. The detection of type IV and VSCC*mec* MRSA in animals have also been reported in other studies (JUHÁSZ-KASZANYITZKY *et al.* 2007; KHANNA *et al.* 2008) and in some cases, with the absence of genes encoding PVL, TSST-1, exfoliatins A and B and enterotoxins (JUHÁSZ-KASZANYITZKY *et al.* 2007; VAN DUIJKEREN *et al.* 2008).

The tested isolates were resistant to oxacillin, amoxicillin/clavulanic acid, cefoxitin, cefotaxime and tetracycline. The resistance to erythromycin, co-trimoxazole, clindamycin, gentamicin and rifampin differ depending on the particular isolate. The data on antimicrobial resistance obtained in different studies vary widely. For instance LEE (2003) has reported MRSA isolates from cattle to be resistant to beta-lactam antibiotics, to clindamycin and erythromycin, and on the other hand, to be highly susceptible to potentiated sulphonamides and rifamycins and often susceptible to tetracycline. The study of JUHÁSZ-KASZANYITZKY *et al.* (2007) has found MRSA isolates from cows to be highly resistant to ampicillin, cephalixin, erythromycin and tetracycline and susceptible to enrofloxacin, gentamicin and potentiated sulphonamides. DE NEELING *et al.* (2007) have reported the detection of MRSA in pigs. Their isolates were susceptible to potentiated sulphonamides and rifamycins and also showed lower resistance to clindamycin and erythromycin, but were all resistant to tetracycline. The resistance is therefore likely to be a result of selective pressure of a specific environment.

## CONCLUSION

The presence of MRSA in basic food production poses a risk of spreading the pathogens to other



animal species, humans involved in animal care and food processing, foodstuffs and consequently to the general population.

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