

Resistance to erythromycin of *Staphylococcus* spp. isolates from the food chain

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ABSTRACT: The aim of this study was to determine both the occurrence and the genetic basis of resistance to erythromycin among 1 235 *Staphylococcus* spp. isolates obtained between 2000 and 2006 from (a) raw milk and meat (1 704 samples), (b) foodstuffs produced from these (451 samples), and (c) contact surfaces at processing plants and dairy farms (363 samples) in the Czech Republic. Isolates were screened by broth microdilution method for resistance to erythromycin and further 11 antimicrobial agents. In addition, isolates were screened by agar dilution (erythromycin range 1–128 mg/l) and D-zone test for inducible resistance to macrolides, lincosamides and streptogramin B (iMLS_B). Forty isolates were found to be either resistant, or intermediate, to erythromycin (3.2% of isolates); of these, more than 50% were identified as *S. epidermidis*. A total of 15 (1.2%) resistant isolates of staphylococci originated from foodstuffs. Resistance mediated by methylation – i.e. iMLS_B-resistance (10 isolates with the *erm*(A) or *erm*(C) gene) and constitutive MLS_B-resistance (one isolate with the *erm*(B) and *erm*(C) genes) – exhibited a significantly high level of resistance to erythromycin with minimum inhibitory concentrations (MIC) of 64 – >128 mg/l (MIC_{mode} = >128 mg/l). In contrast, the efflux mechanism encoded by the *msr*(A) gene (13 isolates; MIC_{range} = 4–128, MIC_{mode} = 128 mg/l), the inactivation mechanisms of resistance encoded by the *mph*(C) gene (three isolates; MIC_{range} = 8–32 mg/l), and/or their combination (13 isolates; MIC_{range} = 4–128, MIC_{mode} = 64 mg/l) led to lower MIC values. The efflux gene *msr*(A) dominated among the erythromycin-resistant isolates (65% of resistant isolates). This first report on the resistance of *Staphylococcus* spp. to erythromycin in the Czech Republic illustrates that, while occurrence was low, isolates from food were nevertheless carriers of *erm*(A), *erm*(B), *erm*(C), *msr*(A) and *mph*(C) genes for resistance to erythromycin and, therefore, represent a potential threat to humans.

Keywords: food safety; inducible MLS_B-resistance; *msr*(A); *mph*(C)

Macrolides are antibiotics widely used for the treatment of human and animal infections. The use of these antibiotics has been accompanied by selection of resistant bacteria, e.g. staphylococci (Schlegelova et al., 2002). Resistant bacteria, or genetic determinants of resistance, can be transmitted from animals to humans via foodstuffs (Perreten et al., 1998; Schlegelova et al., 2004). In addition to pathogenic *Staphylococcus aureus*, potentially pathogenic coagulase-negative staphylo-

cocci (CoNS) have also been identified as carriers of genes for resistance to macrolides (Jensen et al., 1999; Luthje and Schwarz, 2006).

To date, 65 genes have been found to mediate different mechanisms of resistance to macrolides (including target site modification by methylation of rRNA as well as efflux or enzymatic inactivation of macrolides) in both Gram-positive and Gram-negative microorganisms (<http://faculty.washington.edu/marilynr/ermwebA.pdf>, 2007). Among

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these genes, *erm(A)*, *erm(C)* and *msr(A)* have been detected in *Staphylococcus*, alone or in combination, more frequently than other genes (Eady et al., 1993; Lina et al., 1999; Luthje and Schwarz, 2006; Bagcigil et al., 2007). The methylation of rRNA leads to cross-resistance to macrolides, lincosamides and streptogramin B (MLS_B-resistance), which can be either constitutive (cMLS_B) or inducible (iMLS_B) (Weisblum, 1985). High-level resistance to MLS_B antibiotics can be induced by subinhibitory concentrations (0.01–0.1 µg/ml) of erythromycin (Matsuoka et al., 2002).

It is clinically important to differentiate between isolates with iMLS_B resistance encoded by *erm* genes and isolates with efflux-mediated resistance due to the *msr(A)* gene (Leclercq, 2002). Fiebelkorn et al. (2003) described a practical disc diffusion method for the differentiation of erythromycin-resistant isolates and detection of iMLS_B-resistant strains (D-zone test). The therapeutic effect of lincosamides is maintained in erythromycin-resistant strains with the *msr(A)* gene, but the same agents favor the switch from the inducible to the constitutive type of *erm* expression and can be a cause of unsuccessful therapy with lincosamides (Drinkovic et al., 2001).

To date, no study assessing the potential danger of transmitting this type of resistance through foods of animal origin has been carried out in the Czech Republic. Therefore, the primary aim of this study was to critically evaluate the occurrence of erythromycin resistance in staphylococcal isolates from samples related to the human food chain in the Czech Republic. The second aim was to assess the genetic basis of the resistance to erythromycin among the isolates.

MATERIAL AND METHODS

Samples

A total of 2 518 samples (Table 1) were collected between 2000 and 2006 within the Ministry of Agriculture of the Czech Republic's program for monitoring of resistant bacteria and while undertaking research projects in the field of food microbiology. Samples of raw materials and foodstuffs were taken and processed in accordance with CSN ISO 3100-1, CSN EN ISO 707 and CSN EN ISO 6887-2 (Czech Standards Institute, in accordance with ISO international standards). Scrapings taken with an abrasive swab were immediately shaken in

Table 1. Description of samples included in studying the prevalence of erythromycin-resistant *Staphylococcus* in the Czech Republic

Origin of samples	Number of samples	Production facility	Type of sample
Raw milk	1 435	197 milk farms	individual cow and bulk tank milk samples
Scrapings from equipment surfaces at processing plants	242	16 milk farms and 4 dairies	milking equipment; piping and tanks for raw, pasteurized and UHT milk; processing equipment for manufacturing quark, fresh and ripened cheeses
Final milk products	297	5 dairies	milk, UHT milk, cultured milk drinks, quark, butter, butter spread, yogurts, soft and hard cheeses (unflavored), cream cheese
Milk subtotal	1 974		
Raw meat	269		beef, pork and poultry, ready-to-cook meat
Scrapings from equipment surfaces at processing plants	121	5 slaughterhouses and meat processing plants	hanging hooks, saws, cutting tables, steam baths, cutters, sausage and salami fillers, conveyor belts, transport carts
Final meat products	154		meat designated for sale, frankfurters, frankfurters with cheese, sausages, cured long-life meat products and salami from pork, beef and poultry
Meat subtotal	544		
Total	2 518		

10 ml of a with 0.1% peptone (w/v) solution and processed as for analytical samples, again according to CSN EN ISO 6887-2.

Staphylococcal isolates

Samples were cultivated in parallel on Baird-Parker agar (Merck, Darmstadt, Germany) and KRANEP agar (Merck). A maximum of five suspected morphologically dissimilar colonies of *Staphylococcus* were cultivated onto blood agar plates containing 5% sheep blood and identified using the catalase test, oxidase test, bacitracin and furazolidon susceptibility disk diffusion tests, the coagulase test and, biochemically, by the STAPHYtest 24 identification system (Pliva-Lachema, Brno, Czech Republic). Not more than one isolate of the same species from a single sample were included in this study. The isolates were stored in tryptose soy broth (TSB; Oxoid, Basingstoke, UK) supplemented with 20% glycerol at 80°C until studied.

Antimicrobial testing

Determination of minimum inhibitory concentrations (MICs). A total of 1 235 isolates of *Staphylococcus* were examined for resistance to antimicrobial agents. MICs for ampicillin/sulbactam, benzylpenicillin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin, sulphamethoxazole/trimethoprim, teicoplanin, tetracycline and vancomycin were determined using

a standardized microdilution test (Veterinary plate for staphylococci, Trios, Prague, Czech Republic) and, furthermore, for erythromycin (Sigma-Aldrich, Prague, Czech Republic; range 1–128 mg/l) using the agar dilution method in accordance with the Clinical and Laboratory Standards Institute (2006a). The MIC interpretative criteria were based on the recommendations given in document M100-S16 of the Clinical and Laboratory Standards Institute (2006b). *S. aureus* ATCC 25923 served as a reference strain for quality control purposes.

Determination of the inducible type of MLS_B resistance. A total of 40 isolates resistant and intermediate to erythromycin identified on the basis of their MICs were examined using the disc diffusion test (D-zone test) with erythromycin (15 µg) and clindamycin (2 µg) discs (Oxoid) according to the method of Fiebelkorn et al. (2003). A flattening of the zone of growth inhibition around the clindamycin disk in the part adjacent to the erythromycin disk indicated inducible macrolide, lincosamide and streptogramin B (iMLS_B) resistance.

Detection of genes encoding erythromycin resistance

Template DNA was obtained by lysis of cells from a bacterial culture induced by boiling. PCR detection of *erm(A)*, *erm(B)*, *erm(C)*, *msr(A)* and *mph(C)* genes was performed as previously described (Sauer et al., 2008) with a few modifications. PCR cycles consisted of an initial denaturation step (94°C for 5 min) followed by 30 amplification cycles (dena-

Table 2. Primers used for detecting genes encoding resistance to erythromycin among *Staphylococcus* spp. isolates from the food chain

Primer	Sequence (5'→3')	Size of amplicon	References
<i>erm(A)</i> -1 <i>erm(A)</i> -2	GCGGTAAACCCCTCTGAG GCCTGTCCGAATTGG	434 bp	Werckenthin and Schwarz (2000)
<i>erm(B)</i> -1 <i>erm(B)</i> -2	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG	425 bp	Jensen et al. (1999)
<i>erm(C)</i> -1 <i>erm(C)</i> -2	ATCTTTGAAATCGGCTCAGG CAAACCCGTATTCCACGATT	295 bp	Jensen et al. (1999)
<i>msr(A)</i> -1 <i>msr(A)</i> -2	GCAAATGGTGTAGGTAAGACAAC ATCATGTGATGTAAACAAAAT	400 bp	Wondrack et al. (1996)
<i>mph(C)</i> -1 <i>mph(C)</i> -2	GAGACTACCAAGAAGACCTGACG CATACGCCGATTCTCCTGAT	722 bp	Luthje and Schwarz (2006)

turation at 94°C for 60 s, annealing at 51°C [*erm*(A), *erm*(B), *erm*(C)] or 55°C [*msr*(A), *mph*(C)] for 60 s, and extension at 72°C for 60 s) with a final extension at 72°C for 5 min. The sequences of the primers used are given in Table 2.

RESULTS AND DISCUSSION

Prevalence of erythromycin-resistant staphylococcal isolates

Out of a total of 1 235 isolates of *Staphylococcus* spp. of various origins (Table 3), 23 species (including subspecies) were identified: *S. aureus* (560), *S. auricularis* (23), *S. capitis* subsp. *urealyticus* (16), *S. caprae* (7), *S. cohnii* subsp. *cohnii* (5), *S. cohnii* subsp. *urealyticus* (3), *S. epidermidis* (148), *S. felis* (5), *S. haemolyticus* (102), *S. hominis* subsp. *hominis* (18), *S. hyicus* (20), *S. chromogenes* (30), *S. lentus* (1), *S. lugdunensis* (2), *S. muscae* (3), *S. piscifermentans* (4), *S. saprophyticus* subsp. *bovis* (21), *S. saprophyticus* subsp. *saprophyticus*

(48), *S. sciuri* subsp. *sciuri* (7), *S. schleiferi* subsp. *coagulans* (3), *S. simulans* (16), *S. warneri* (89), and *S. xylosus* (93), as well as 11 isolates of nontypeable coagulase-negative staphylococci.

In selecting interpretative criteria for evaluating resistance, it was necessary to consider isolates as carriers of resistance genes that can cause problems, and particularly in the therapy of humans. Therefore, we selected the criteria for clinical laboratories cited by the Clinical and Laboratory Standards Institute (CLSI) (2006b) to interpret resistance. In this study, 40 isolates were found to be resistant or intermediate to erythromycin (3.2% of isolates, Table 3). Of these, more than 50% were identified as *S. epidermidis* (21 isolates, Table 4). Other species found were *S. aureus* (8 isolates), *S. haemolyticus* (5 isolates), *S. xylosus* (two isolates), *S. warneri* (one isolate), and three isolates that could not be identified to species level by the tests applied and are therefore referred to as coagulase-negative staphylococci (CoNS).

Raw milk displayed a rather low level of contamination with erythromycin-resistant isolates relative

Table 3. Prevalence of various *Staphylococcus* spp. genes encoding resistance to erythromycin in isolates from milk- and meat-related samples

Origin of samples and isolates	Resistance to erythromycin ^a						
	Number of samples	Number of isolates	Number of isolates (%)	Resistance genes			
				<i>erm</i>	<i>msr</i> (A)	<i>mph</i> (C)	<i>msr</i> (A) <i>mph</i> (C)
Raw milk	1 435	638	13 (2.04)	4	3	0	6
Scrapings from equipment surfaces at processing plants	242	117	6 (5.13)	3	0	1	2
Final milk products	297	263	8 (3.04) ^b	0	3	2	3
Milk subtotal	1 974	1 018	27 (2.65)	7	6	3	11
Raw meat	269	111	6 (5.41)	3	3	0	0
Scrapings from equipment surfaces at processing plants	121	22	0	0	0	0	0
Final meat products	154	84	7 (8.33) ^c	1	4	0	2
Meat subtotal	544	217	13 (5.99)	4	7	0	2
Total	2 518	1 235	40 (3.24)	11	13	3	13

^aintermediate and resistant isolates to erythromycin (MIC ≥ 4 mg/l)

^bfinal milk products contaminated with erythromycin-resistant isolates of *Staphylococcus* spp.: Eidam cheese 1×, Gouda cheese 2×, pasteurized milk 3×, butter 1×, butter spread 1×

^cfinal meat products that were contaminated with erythromycin-resistant isolates of *Staphylococcus* spp.: frankfurter 1×, sausages 4×, frankfurter with cheese 2×

to the total number of isolates from milk (2.04%; Table 3). Therefore, a lower frequency of erythromycin-resistant staphylococci was also expected in final products, which were manufactured only from pasteurized milk (73–76°C, 15–20 s). However, the relative number of resistant isolates increased to 3.04%. Also surprising was the higher number of erythromycin-resistant isolates from the surfaces of milk plant equipment (5.13%), all of which were *S. epidermidis*. *S. epidermidis* has been shown to have the ability to adhere to stainless steel surfaces (Moretro et al., 2003), and a correlation between resistance to antimicrobial agents and the ability to create biofilms has been shown particularly in human medicine (Klingenberg et al., 2005). On the basis of these findings, we can assume that the final milk products in the present study were contaminated secondarily, through strains released from the surfaces of the processing equipment.

Significantly more erythromycin-resistant isolates were extracted from raw meat (Fisher's test; $P < 0.05$) than from raw milk (5.41% versus 2.04%). Further, final meat products were contaminated more often in comparison with milk products (8.33% versus to 3.04%), but that difference was not statistically significant (Fisher's test; $P > 0.05$). In the meat processing plants, no processing steps were included that would eliminate with certainty all microorganisms during the processing of raw meat. It is more likely, therefore, that contamination by microorganisms was caused by transfer from animals, as opposed to transfer from the surfaces of processing equipment, since erythromycin-resistant staphylococci were not isolated from the processing equipment and, in general, the number of isolates in this group was low (22 isolates; Table 3).

Comparable studies that examined resistance to erythromycin of *Staphylococcus* isolates, albeit directly from clinical cases of animal illnesses, indicate an occurrence of 7.4% erythromycin-resistant CoNS isolates from cows suffering from subclinical mastitis (Luthje and Schwarz, 2006), and in 26.3% (31 of 118) of samples of clinically isolated staphylococci from poultry (Aarestrup et al., 2000). Resistance to erythromycin occurred in 38% of isolates of *S. aureus* from pigs, but not among another 300 animals (equal numbers of dogs, horses and cows) that were a part of the same study (Bagcigil et al., 2007). Based upon both these results and our results, we believe the prevalence and penetration of erythromycin-resistant staphylococci in the food

chain is not high, although not negligible. A higher risk for transfer of resistant isolates was shown for final meat products in the Czech Republic than for milk (Table 3).

Analysis of the genetic basis of erythromycin-resistant isolates

At least one of the genetic determinants of resistance was detected in all erythromycin-resistant isolates in this study (MIC_R range 8 to >128 mg/l). The inducible phenotype of resistance to MLS_B antibiotics was detected in 10 isolates (Table 4) with susceptibility to clindamycin ($MIC_{CLI} = 0.03$ – 0.25 mg/l). The inducibly expressed genes *erm(A)* and *erm(C)* were present alone (both in five isolates), while in one isolate of *S. haemolyticus* the *erm(C)* gene was detected in combination with the *erm(B)* gene. This isolate was resistant to macrolides and lincosamides as identified on the basis of MIC values for erythromycin and clindamycin of >128 mg/l and 8 mg/l, respectively. The constitutive phenotype of resistance corresponded in this isolate with the constitutive expression of the *erm(B)* gene, which is indicative for staphylococci (Lina et al., 1999). The simultaneous presence of more than one *erm* gene in the genome is possible and has previously been detected in staphylococci (Eady et al., 1993; Jensen et al., 1999; Luthje and Schwarz, 2006).

In addition to high resistance to erythromycin, which significantly differentiated *erm* positive isolates ($MIC_{90} = >128$ mg/l), resistances to at least one other antimicrobial agent were also noted (Table 4). Multiple resistance to erythromycin, penicillin, and tetracycline, or in combination with other resistance phenotypes, was noted in seven isolates (64.0%). High concurrent resistance to other antimicrobial agents has also been reported by other authors, such as Leclercq (2002), and it may indeed be characteristic for this type of resistance. In this study, however, we could not confirm the correlation reported for *erm(A)* genes, constitutive expression and resistance of staphylococci strains to methicillin, or that for *erm(C)* genes, inducible expression and sensitivity to methicillin (Lina et al., 1999).

Twenty-six isolates resistant to erythromycin carried the *msr(A)* gene, which codes for an ATP-dependent efflux pump (Table 4). This gene occurred in the studied staphylococci more often

Table 4. Comparison of MIC values for erythromycin (ERY) and clindamycin (CLI) with genes for resistance to erythromycin and phenotype of resistance in 40 isolates of *Staphylococcus* spp.

Species	Origin of sample	MIC (mg/l)		Gene	Phenotype of resistance ^a
		ERY	CLI		
<i>S. aureus</i>	raw milk	> 128	0.25	<i>erm(A)</i> _{ind} ^b	PEN-TET-ERY
<i>S. haemolyticus</i>	raw milk	> 128	0.25	<i>erm(A)</i> _{ind}	PEN-TET-ERY
<i>S. aureus</i>	raw meat	> 128	0.06	<i>erm(A)</i> _{ind}	CIP-ERY
<i>S. aureus</i>	raw meat	> 128	0.06	<i>erm(A)</i> _{ind}	CIP-ERY
<i>S. haemolyticus</i>	meat product	64	0.25	<i>erm(A)</i> _{ind}	PEN-TET-ERY
CoNS	raw milk	> 128	0.06	<i>erm(C)</i> _{ind}	PEN-ERY
<i>S. epidermidis</i>	scraping	> 128	0.06	<i>erm(C)</i> _{ind}	CIP-ERY
<i>S. epidermidis</i>	scraping	> 128	0.03	<i>erm(C)</i> _{ind}	PEN-COT-ERY
<i>S. epidermidis</i>	scraping	> 128	0.12	<i>erm(C)</i> _{ind}	PEN-TET-COT-ERY
<i>S. warneri</i>	meat product	> 128	0.25	<i>erm(C)</i> _{ind}	OXA-COT-ERY
<i>S. haemolyticus</i>	raw milk	> 128	8	<i>erm(C)</i> , <i>erm(B)</i>	PEN-COT-ERY-CLI
<i>S. epidermidis</i>	raw milk	128	0.06	<i>msr(A)</i>	PEN-ERY
<i>S. epidermidis</i>	raw milk	128	0.06	<i>msr(A)</i>	COT-ERY
CoNS	raw milk	32	0.50	<i>msr(A)</i>	PEN-ERY
<i>S. epidermidis</i>	milk product	128	0.06	<i>msr(A)</i>	PEN-ERY
<i>S. epidermidis</i>	milk product	128	0.06	<i>msr(A)</i>	PEN-ERY
CoNS	milk product	128	0.03	<i>msr(A)</i>	PEN-ERY
<i>S. epidermidis</i>	raw meat	128	0.25	<i>msr(A)</i>	TET-ERY
<i>S. aureus</i>	raw meat	64	0.25	<i>msr(A)</i>	ERY
<i>S. aureus</i>	raw meat	64	0.06	<i>msr(A)</i>	ERY
<i>S. aureus</i>	meat product	4	0.06	<i>msr(A)</i>	PEN-COT-ERY
<i>S. epidermidis</i>	meat product	128	0.25	<i>msr(A)</i>	PEN-TET-ERY
<i>S. epidermidis</i>	meat product	128	0.06	<i>msr(A)</i>	PEN-ERY
<i>S. epidermidis</i>	meat product	128	0.06	<i>msr(A)</i>	COT-ERY
<i>S. haemolyticus</i>	raw milk	64	0.06	<i>mph(C)</i> , <i>msr(A)</i>	COT-ERY
<i>S. epidermidis</i>	raw milk	64	0.50	<i>mph(C)</i> , <i>msr(A)</i>	OXA-TET-ERY
<i>S. epidermidis</i>	raw milk	64	0.25	<i>mph(C)</i> , <i>msr(A)</i>	PEN-OXA-TET-ERY
<i>S. epidermidis</i>	raw milk	32	0.25	<i>mph(C)</i> , <i>msr(A)</i>	PEN-OXA-AMS-TET-ERY
<i>S. epidermidis</i>	raw milk	64	0.25	<i>mph(C)</i> , <i>msr(A)</i>	PEN-OXA-TET-ERY
<i>S. epidermidis</i>	raw milk	64	0.25	<i>mph(C)</i> , <i>msr(A)</i>	PEN-OXA-AMS-TET-ERY
<i>S. epidermidis</i>	scraping	128	0.06	<i>mph(C)</i> , <i>msr(A)</i>	PEN-OXA-TET-COT-ERY
<i>S. epidermidis</i>	scraping	32	0.06	<i>mph(C)</i> , <i>msr(A)</i>	ERY
<i>S. epidermidis</i>	milk product	16	0.06	<i>mph(C)</i> , <i>msr(A)</i>	ERY
<i>S. haemolyticus</i>	milk product	32	0.06	<i>mph(C)</i> , <i>msr(A)</i>	COT-ERY
<i>S. aureus</i>	milk product	8	0.06	<i>mph(C)</i> , <i>msr(A)</i>	PEN-ERY
<i>S. aureus</i>	meat product	4	0.06	<i>mph(C)</i> , <i>msr(A)</i>	TET-ERY
<i>S. epidermidis</i>	meat product	64	0.06	<i>mph(C)</i> , <i>msr(A)</i>	COT-ERY
<i>S. epidermidis</i>	scraping	16	0.06	<i>mph(C)</i>	COT-ERY
<i>S. xylosum</i>	milk product	32	0.06	<i>mph(C)</i>	ERY
<i>S. xylosum</i>	milk product	4	0.03	<i>mph(C)</i>	ERY

^aantimicrobial agents: AMS = ampicillin-sulbactam; CIP = ciprofloxacin; COT = sulphamethoxazole-trimethoprim; ERY = erythromycin; OXA = oxacillin; PEN = benzylpenicillin; TET = tetracycline

^binducible expression of *erm* genes

than other genes, either alone (13 isolates) or in combination with the *mph(C)* gene, which codes for an erythromycin inactivating phosphotransferase (13 isolates). Surprisingly, the simultaneous presence of the *msr(A)* and *mph(C)* genes led to lower MIC values ($MIC_{mode} = 64$ mg/l) than noted for isolates that carried only the gene *msr(A)* ($MIC_{mode} = 128$ mg/l). As previously described, the genes *msr(A)* and *mph(C)* were usually physically linked in staphylococci (Luthje and Schwarz, 2006). Our results indicate that the *msr(A)* gene occurs more often in environments associated with animals than has been previously indicated in, for example, isolates from humans (Eady et al., 1993). The higher occurrence is evidenced by findings of genes in isolates extracted from the genera *Streptococcus*, *Enterococcus*, *Corynebacterium* and *Pseudomonas*, all of which are highly homologous with the *msr(A)* gene in the case of *Staphylococcus* (99 to 100%) (Ojo et al., 2006).

The *mph(C)* gene was detected in 13 isolates in combination with *msr(A)* genes, as well as independently of *msr(A)* in two isolates of *S. xyloso* ($MIC = 4$ and 32 mg/l) and in one isolate of *S. epidermidis* ($MIC = 16$ mg/l). Resistance to other antimicrobial agents was not noted in these cases. This gene has been detected in *S. aureus*, and only in connection with the *msr(A)* and *erm* genes (Matsuoka et al., 1998, 2002). Our data are in agreement with the results of the study by Luthje and Schwarz (2006), who detected the gene independently in two isolates, *S. equorum* and *S. xyloso*, and with MIC values for erythromycin of 8 and 16 mg/l, respectively.

The *msr(A)* and *mph(C)* genes, independently or in combination, were detected also in three isolates (*S. aureus* 2×, *S. xyloso* 1×; Table 4) with MIC to erythromycin of 4 mg/l, which is upper boundary of the intermediate category. Repeated determination of MIC values for these isolates excluded the possibility of an error in performing the test.

CONCLUSION

Based upon the results of this study, we can state that, in the Czech Republic, the occurrence of *Staphylococcus* isolates resistant to erythromycin was low in milk from cattle and in foods of animal origin. Although the findings are not alarming, this first report on the resistance of *Staphylococcus* spp. to erythromycin in the Czech Republic indi-

cates that isolates from foodstuffs were carriers of *erm(C)*, *msr(A)* and/or *mph(C)* genes for resistance to erythromycin and, therefore, represent a potential threat to humans. A higher prevalence of such the isolates was found in meat products when compared with milk products. The results from this study indicate that the *msr(A)* gene occurs more often in staphylococci from animal environments than previously presented in clinical isolates from humans.

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